# ENDOCRINOLOGY

THE BULLETIN OF
THE ASSOCIATION FOR THE STUDY
OF INTERNAL SECRETIONS

VOLUME 22 FEBRUARY, 1938 NUMBER 2

## A STUDY OF THE MECHANISM OF EDEMA ASSOCIATED WITH MENSTRUATION

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The regular occurrence of generalized edema associated with menstruation has been reported by Thomas (1), Sweeney (2), Molnar and Gruber (3) Atkinson and Ivy (4). An increase in body weight and a swelling of the abdomen, hands and feet were noted by Eufinger and Spiegler (5), Okey and Stewart (6), and Sweeney (2), in approximately one-third of normal women at or about the time of menstruation. In most instances a rapid reduction in weight occurred during menstruation. Evidence of a disturbance in water metabolism at the time of menstruation was described by Heilig (7), and Peterson and Milles (8). Heilig (7) observed a delayed excretion of water at the time of menstruation as compared to the intermenstrual period. Peterson and Milles (8), using the 'blister technic' noted an alteration in the permeability of the capillaries of the skin of normal women during menstruation.

Preliminary experiments (9) indicated that in normal dogs subcutaneous injections of estrone and progesterone produced a retention of sodium, chloride and water. It seemed possible that the cyclical retention of sodium, chloride, and water which was observed premenstrually in both normal subjects and patients might be related to the cyclical variation in the secretion of the sex hormones. Smith and Smith (10) noted that in normal women the highest concentration of estrogenic substance in the urine occurred approximately 12 days preceding menstruation (at or about the time of ovulation). A second increase in the excretion of estrogenic substance occurred during the premenstrual period. In previous studies (2, 5, 6) little attention was given to changes in body weight which may have occurred during the intermenstrual period. In this study, the observation that injections of estrone as well as progesterone resulted in sodium, chloride and water retention in normal dogs

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suggested the advisability of investigating the fluctuation in body weight which might occur in normal women at or near the time of ovulation as well as during the premenstrual phase of the cycle.

## PROCEDURE AND RESULTS

*A. Electrolyte excretion in dogs, as affected by sex hormones.* Normal male and female dogs were injected with crystalline preparations of several of the sex hormones (estrone, estradiol, progesterone and testosterone). The dogs, weighing approximately 10 kg., were maintained in metabolism cages under uniform conditions (11) with a constant fluid and mineral intake. They were catheterized at the beginning and at the end of each 24-hour period.

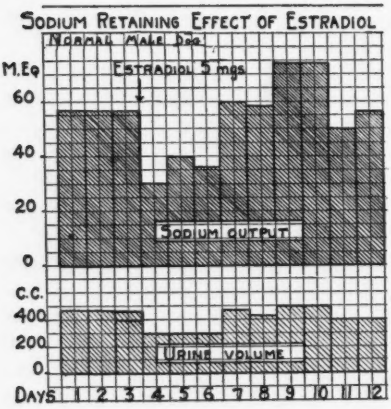


Fig. 1

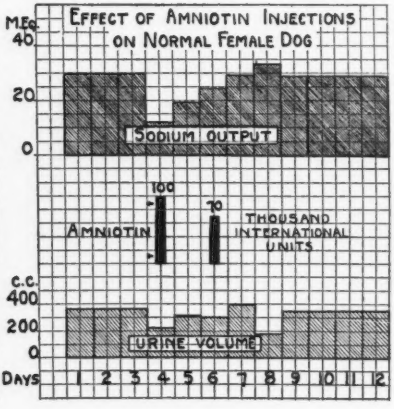


Fig. 2

Fig. 1. Single subcutaneous injection of 5 mg. of estradiol.
Fig. 2. A single subcutaneous injection of amniotin was made on the 4th and 6th day of the experiment.

Under these conditions a constant 24-hour excretion of sodium and chloride ($\pm$ 3 m. eq.) was obtained (11). The dogs were injected with crystalline preparations of several of the sex hormones, and the effect on the 24-hour renal excretion of electrolytes was observed. The injection of 5 mg. of alpha estradiol[2] into a normal male dog resulted in a marked and prolonged (48 to 72 hours) decrease in the renal excretion of sodium and chloride, a reduction in urine volume and an increase in body weight of 0.4 kg. (fig. 1). Five days subsequent to the injection of estradiol a 'rebound' effect was observed in which the increased excretion of sodium approximated the quantity previously retained. This 'rebound' effect was observed regularly in dogs injected with adrenal cortical hormone (11).

Amniotin[3] (70,000 and 100,000 I.U.) injected into a normal female dog also produced a retention of sodium, a reduction in urine volume and an increase in body weight of 0.3 kg. (fig. 2). The retention of sodium and

[2] Crystalline alpha estradiol and progesterone were kindly supplied by Dr. O. Wintersteiner of Columbia University, New York.
[3] The amniotin used in this study was supplied by Dr. J. A. Morrell of the E. R. Squibb Company, New York.

water occurred, although true estrus was not induced. Injection of amniotin (25,000 to 100,000 I.U.) into male dogs produced similar changes in the renal excretion of sodium.

A study of several of the sex hormones revealed the fact that a 'sodium-retaining' property was common to all. In table 1 the crystalline preparations of sex hormones[4] have been arranged according to the 'sodium-retaining' effect. The basis of comparison was 10 mg. of crystalline material.

In addition to the sex hormones, injections of pregnanediol have been shown to exert a 'sodium-retaining' effect on the intact animal (table 1). Pregnanediol is known not to possess any of the sex hormone activities of progesterone, from which it is supposedly derived (12, 13). All of the sex hormones and closely related sterol compounds thus far tested were found to exert a 'sodium-retaining' effect, as measured by a decrease in the renal

TABLE 1. ASSAY OF 'SODIUM-RETAINING PROPERTY' OF THE SEX HORMONES

| Substance[1] | Quantity mg. | Assay Dog Units[2] |
|---|---|---|
| Estradiol (crystalline) | 10 | 700 |
| Progesterone (crystalline) | 10 | 400 |
| Estrone (crystalline) | 10 | 200–300 |
| Pregnanediol[3] (crystalline) | 10 | 140 |
| Testosterone (crystalline) | 10 | 80 |
| Testosterone (Propionic acid ester) | 10 | 25–40 |

[1] Single subcutaneous injection of material taken up in corn oil.
[2] 'Dog Units' refers to the quantity of adrenal cortical hormone (standardized by the manufacturers on adrenalectomized dogs) necessary to produce an equivalent sodium retention.
[3] Not a sex hormone, but closely related chemically to progesterone.

excretion of sodium in the normal dog. Observations on patients with Addison's disease and adrenalectomized dogs (14) confirmed the effectiveness of estrogenic substances (estradiol, amniotin) in producing a retention of sodium, chloride and water.

*B. Weight changes in women during the menstrual cycle.* The fluctuation in the body weight of 50 normal women was observed during a period of 35 days. This period represented one complete menstrual cycle. The ages of the subjects, who were nurses and dietitians, varied between 19 and 36 years, the average age being 23.6 years. The subjects were weighed on the same balance each morning immediately prior to breakfast. The uniform and shoes worn by each subject were of constant weight. No attempt was made to control either the total fluid or food intake. In general, the food provided for each member of the group was similar. The onset and duration of menstruation were noted, as well as the occurrence of any unusual signs and symptoms such as headache, swelling of the abdomen, hands and feet, increased appetite and thirst. This study was conducted in Baltimore during the months of April and May, 1937. During the period of observation, the mean daily atmospheric temperature varied between 76.9° and 56.8° F. The relative humidity, measured at noon, varied between 82 and 19 per cent.

[4] The preparations of testosterone used in this study were kindly supplied by Dr. Max Gilbert of the Schering Corporation, Bloomfield.

In the group of 50 normal subjects, 24 (48 per cent) were observed to gain 1 kg. (2.2 lb.) or more during the premenstrual period (table 2). In addition, 9 subjects gained weight during the premenstrual period, but a subsequent loss occurred prior to the onset of menstruation (table 2). In many of the subjects the onset of menstruation was characterized by a rapid loss of weight (table 2). These data confirm the observations of Eufinger and Spiegler (5), Okey and Stewart (6) and Sweeney (2).

During the intermenstrual period, however, 38, or 76 per cent of the subjects, were observed to have a marked fluctuation (temporary gain) in

TABLE 2. FLUCTUATION IN BODY WEIGHT DURING THE MENSTRUAL CYCLE (50 NORMAL SUBJECTS)

| Period | Gain[1] | | Loss[1] | | Temporary Gain or Loss[1] | | | | No Significant Change | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | A[2] | | B[3] | | | |
| | No. of Subjects | % | No. of Subjects | % | No. of Subjects | % | No. of Subjects | % | No. of Subjects | % |
| Premenstrual (7–10 days) | 24[4] | 48 | 2 | 4 | 9 | 18 | 0 | | 15 | 30 |
| Menstrual | 3 | 6 | 22[4] | 44 | 4 | 8 | 1 | 2 | 20 | 40 |
| Post-Menstrual (3–5 days) | 3 | 6 | 15[4] | 30 | 1 | 2 | 0 | | 31 | 62 |
| Intermenstrual | 0 | | 2 | 4 | 38[4] | 76 | 0 | | 10 | 20 |

[1] Represents an alteration of 1 kg. or more.
[2] Refers to an initial increase in weight with a subsequent loss occurring during the same period.
[3] Refers to an initial decrease in weight with a subsequent gain occurring during the same period.
[4] Refer to text for description.

TABLE 3. SUMMARY OF THE SIGNS AND SYMPTOMS OBSERVED DURING THE PREMENSTRUAL PERIOD (50 normal subjects)

| Signs and Symptoms | Number of Subjects | Per Cent of Group |
|---|---|---|
| Swelling of abdomen[1] | 33 | 66 |
| Increased appetite | 11 | 22 |
| Increased thirst | 12 | 24 |
| Headache | 21 | 42 |

[1] Occasionally associated with swelling of the feet and hands.

weight (1 kg. or more) at or about the time of ovulation (table 2). Estrin and pregnanediol determinations performed in 3 subjects (*C*) indicated that the intermenstrual increase in weight occurred at or about the time of ovulation. In the large group of normal subjects, the exact relationship of the intermenstrual weight change to the time of ovulation could not be determined. Since the increase in body weight was noted from 8 to 15 days preceding menstruation, it is probable that ovulation occurred in a high per cent of normal subjects during the period of weight increase. The increase and subsequent loss of weight which occurred during the intermenstrual period were not always followed by an increase in weight during the premenstrual period. Approximately two thirds of the subjects who gained weight during the premenstrual period noted swelling of the abdomen, and increased appetite

and thirst. One-third of the subjects, in whom no significant weight increase occurred, also experienced the same signs and symptoms. Headache was frequently observed to occur immediately preceding or during menstruation (table 3). The increased intake of food and water as a result of an increased appetite and thirst contributed materially to the total gain in body weight observed in some subjects during the premenstrual period.

*C. Electrolyte excretion in women during the menstrual cycle.* The daily renal excretion of electrolytes was followed in 4 normal subjects during a part or all of one menstrual cycle. In 1 of the normal subjects, the renal

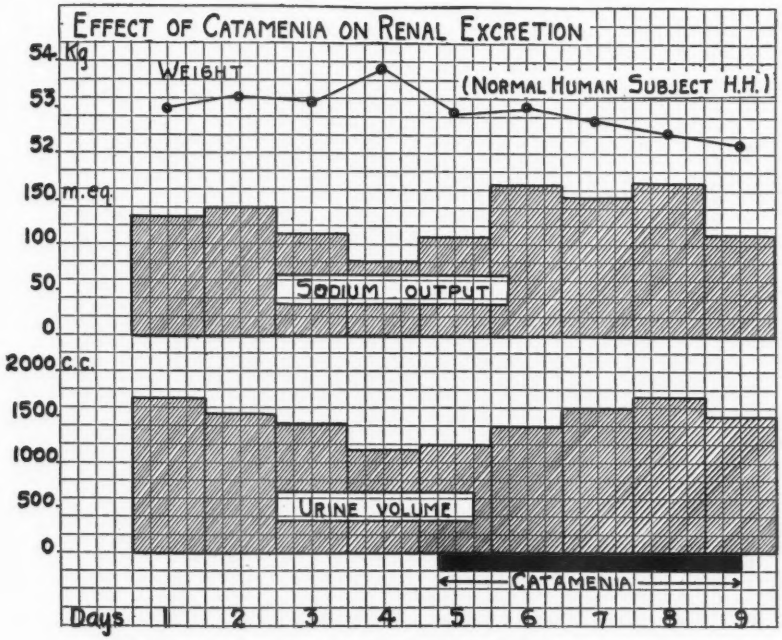


Fig. 3. The subject was provided with a constant diet and fluid intake. The diet contained 110.2 m.eq. of sodium, 115.9 m.eq. of chloride and 61.5 m.eq. of potassium.

excretion of electrolytes and estrogenic substance was determined throughout an entire menstrual cycle, and, in two patients who were known to gain weight immediately preceding menstruation, the renal excretion of electrolytes, estrogenic substance, and pregnanediol was determined during the menstrual cycle.

These two patients (*D. R.* and *E. M.*) and 4 normal female subjects (*H. H., K. K., J. J.* and *D. W.*) were provided with a diet which was constant in caloric value, mineral and water content. The diet of the two obese patients (*D. R.* and *E. M.*) was limited to 1000 cal. per day for the purpose of accomplishing weight reduction. The normal subjects received a diet of caloric value sufficient to maintain weight, and the daily fluid intake was constant. The subjects were maintained on a constant regimen during a part or all of one menstrual cycle, and body weight was determined daily before breakfast. The

preparation and analysis of the diet have been described elsewhere (15). Twenty-four hour urine specimens were collected and preserved with toluene. The urine was analyzed for its sodium, potassium, chloride, inorganic phosphate and total nitrogen content (16). Estrin was determined on 3-day pooled urine specimens throughout an entire menstrual cycle (10). Similar studies were made on 1 normal male subject. In 2 patients (*D. R.* and *E. M.*) urinary pregnanediol excretion was determined for a 10-day period immediately preceding menstruation (12).

The renal excretion of sodium and chloride was decreased appreciably in 3 of 4 normal subjects (*H. H.*, *J. J.*, and *D. W.*) during the 3 days immediately preceding menstruation. It is of interest to note that the changes in chloride excretion confirm the observations of Pucher et al (17). In 2 of the subjects (*H. H.* and *D. W.*) the retention of sodium and chloride was associated with a gain in body weight and a slight increase in the excretion of potassium (fig. 3, subject *H. H.*, fig. 4, subject *D. W.*). The onset of menstruation in subjects *H. H.*, *J. J.* and *D. W.* was marked by an increase in the excretion of sodium, chloride and water, and a reduction in body weight. During menstruation, the potassium excretion was reduced in subjects *H. H.* and *D. W.* and increased in subject *J. J.* In the fourth subject, *K. K.*, a diuresis was observed 24 hours immediately preceding the onset of menstruation. This diuresis was associated with an increase in the excretion of sodium, chloride and potassium.

A marked retention of sodium and chloride was observed to occur in patients *D. R.* and *E. M.* during the premenstrual period (patient *D. R.*, fig. 5). No gain in weight was noted at this time, but during the period of sodium retention the patients maintained body weight, failing to show the regular daily weight loss which had occurred as a result of the restricted diet (fig. 5, patient *D. R.*). Patient *D. R.* experienced a decided increase in appetite during the premenstrual period. Previously, when no dietary restrictions were imposed, these patients had gained as much as 2 kg. immediately preceding the onset of menstruation. In both *D. R.* and *E. M.* the onset of menstruation was characterized by weight loss and an increased renal excretion of sodium, chloride and water.

The urinary excretion of pregnanediol (12) was determined[5] during a 10-day period immediately preceding menstruation in *D. R.* and *E. M.* (fig. 5, patient *D. R.*). Venning and Browne (13) consider pregnanediol to be an excretion product of progesterone, and that its presence in the urine during the latter half of the menstrual cycle may be taken as evidence of a functioning corpus luteum. Pregnanediol excretion appears to bear a quantitative relationship to the secretion of progesterone. In both patients *D. R.* and *E. M.* approximately 15 mg. of pregnanediol was recovered during the entire premenstrual period. Not enough determinations of pregnanediol have been made to permit quantitative evaluation of the results obtained in these patients. The values are presented as evidence that a functioning corpus luteum was present during the latter half of the menstrual cycle, and that the menstrual cycle

[5] The authors are indebted to Dr. E. B. Astwood of the Johns Hopkins Hospital for both the determinations of pregnanediol and the supply of crystalline pregnanediol used in this study.

may thus be considered to have been a normal one. During the same premenstrual period the excretion of estrogenic substance was found to be approximately 600 R.U. in the case of *D. R.* and approximately 400 R.U. in the case of *E. M.*

The changes which occurred in the renal excretion of electrolytes and estrogenic substance[6] were followed during an entire menstrual cycle in normal subject *D. W.* (fig. 4) and patient *D. R.* (fig. 5). Sodium and chloride were retained at the time of ovulation as well as premenstrually. Ovulation was indicated by the increase and subsequent decrease of estrogenic substance in the urine and the appearance of pregnanediol (fig. 5). The fluctuation in the excretion of sodium, chloride and water at the time of ovulation differed only quantitatively from the changes noted during the premenstrual period.

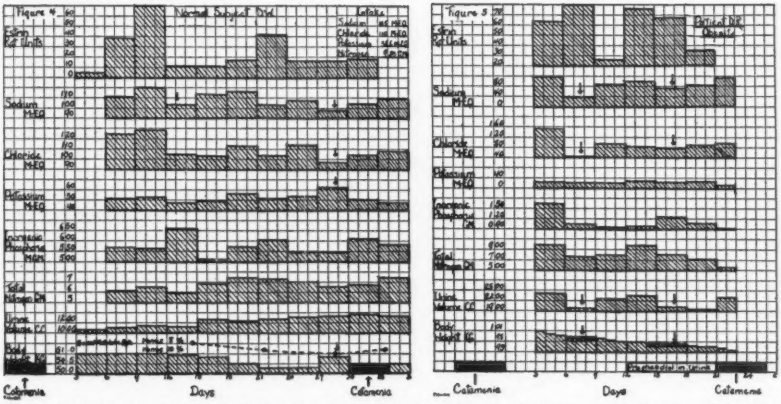

Fig. 4. Renal excretion of electrolytes and estrogenic substance during a menstrual cycle. The intake was: sodium 105 M.E.Q., chloride 110 M.E.Q., potassium 58.6 M.E.Q., and nitrogen 9.83 gm.

Fig. 5. Renal excretion of electrolytes and sex hormones during a menstrual cycle. The diet was of constant mineral content. Calories were restricted to 1000 per day.

Shortly after ovulation an increase in urine volume, sodium and chloride excretion was noted.

## DISCUSSION

In these studies, particular significance has been given to the values obtained for sodium and chloride, since the renal excretion of these substances has been shown to represent approximately 92 to 95 per cent of the total excretion in normal subjects under constant conditions (15). Less significance should be attached to the values representing the renal excretion of potassium, phosphate and nitrogen, since appreciable quantities of these substances (10 to 25 per cent) may be excreted in the feces.

In normal subjects the loss of sodium, chloride and potassium in the menstrual flow does not appreciably affect the balance of these electrolytes in the body. Barer and Fowler (18) record a blood loss of 50.5 cc. as the mean observed in 100 normal women. Assuming the concentration of sodium

[6] Estrogenic substance in the urine was determined in the Endocrinology Laboratory of the Johns Hopkins Hospital according to the method of Smith and Smith (10).

to be 90 m. eq. per liter of whole blood, chloride 90 m. eq. and potassium 50 m. eq., a blood loss of 50 cc. would represent a total loss of 4.5 m. eq. of sodium and chloride, and 2.5 m. eq. of potassium. This very small loss should be divided by at least 2 or 3 days, since only a part of the blood loss occurs during a single day. In subject *D. W.*, direct determination of the total sodium, chloride and potassium loss occasioned by the menstrual flow gave values of approximately 7 m. eq. of sodium and chloride and 4.5 m. eq. of potassium.

For purpose of control, a normal male subject (*W. T.*, aged 30) was provided with a constant diet and fluid intake. The diet contained 105 m. eq. of sodium, 110 m. eq. of chloride, 59.5 m. eq. of potassium and 9.86 gm. of nitrogen. The daily renal excretion of these substances was determined. Estrin determinations were made on 7-day pooled specimens of urine.

No fluctuation in body weight was observed throughout the study (35 days), although there was a gradual loss of weight, amounting to a total of 0.7 kg. Estrin determinations were quite constant (less than 3 R.U. per day). An appreciable daily fluctuation (15 to 35 m. eq.) of sodium and chloride occurred with some regularity throughout the period of observation. The urine volume varied simultaneously with the changes in sodium and chloride. Total nitrogen, phosphate, potassium, body weight and B.M.R. did not appear to be significantly affected. The fluctuations in the excretion of sodium, chloride and water could not be correlated with any known changes in environment.

Normal male and female dogs injected with estrone, estradiol, progesterone, testosterone and pregnanediol were found to retain sodium, chloride and water in all instances. The relative effectiveness of 10 mg. quantities of these substances was determined. The close chemical relationship between the sex hormones and compounds derived from the adrenal cortex is known. It is felt that the hormone of the adrenal cortex and the sex hormones may be similar not only in chemical structure, but also in at least one physiological effect, i.e., the property of inducing salt and water retention.

The fluctuation in the body weight of 50 normal women was observed during a period of 35 days. Twenty-four of the subjects (48 per cent) were observed to gain 1 kg. or more during the premenstrual period; 38 of the subjects (76) per cent) gained 1 kg. or more at or about the time of ovulation. Menstruation and the postmenstrual period were associated with weight loss in approximately one-half the subjects.

A striking symptom, noted in approximately 25 per cent of the group of 50 normal subjects, premenstrually, was the increase in appetite and thirst. Apparently an increase in food and water consumption added greatly to the total gain in body weight observed in some of the subjects at this time. Restriction of food and fluid intake in two patients was found to be an effective method of preventing any considerable increase in weight during the premenstrual period.

The renal excretion of electrolytes was studied during a part or all of one menstrual cycle in 2 patients, who were known to gain weight during the premenstrual phase of the cycle, and in 4 normal subjects. A retention of

sodium, chloride and water was observed during the premenstrual period in both patients, and in 3 of the 4 normal subjects. In 1 patient and 1 normal subject, the renal excretion of electrolytes and estrogenic substance was followed throughout a complete menstrual cycle. In both instances, the increased output of estrin at the time of ovulation was associated with a sodium, and chloride retention. Pregnanediol determinations were made on the urine of 2 patients. The presence of pregnanediol in both instances was considered additional evidence of a functioning corpus luteum, and hence a presumably normal cycle. Too few determinations of pregnanediol have been made to allow interpretation of the values obtained in this study. A normal male subject was found to have a constant small quantity of estrogenic substance in the urine. No fluctuation in body weight was observed. A small, but definite cyclical variation in the renal excretion of sodium, chloride and water was observed, for which phenomenon no explanation has as yet been found.

## SUMMARY

The injection of crystalline preparations of estrone, progesterone, pregnanediol and testosterone induced the retention of sodium, chloride and water in normal dogs. Balance studies in human subjects demonstrated a retention of sodium, chloride and water during the intermenstrual as well as the premenstrual phase of the cycle. The onset of menstruation was associated with an increased renal excretion of sodium, chloride and water. Normal subjects, in whom no dietary restrictions were imposed, were observed to gain weight during the intermenstrual as well as during the premenstrual phase of the cycle. An increase in appetite and thirst was a striking symptom noted during the premenstrual period. The increase in the secretion of sex hormones and the increase in appetite and thirst appear to be contributing factors in body weight gain, which occurs during the menstrual cycle.

The kind coöperation of the School of Nursing, Johns Hopkins Hospital, is acknowledged.

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# EXPERIMENTAL DEVELOPMENT OF THE MAMMARY GLAND OF THE MONKEY[1]

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It has been found that the injection of ovarian hormones develops the mammary glands of several species of laboratory animals. In some species such as the guinea pig, a complete development occurs in animals receiving only estrogenic chemicals, while in the rabbit estrone and progesterone are both required (1, 2). Only mammary ducts developed in rabbits receiving estrogenic hormones, while complete growth of the glands occurred in animals receiving, in addition, extracts of the corpus luteum. Localized nodules of alveoli may occur in the mammary glands of male or female mice of certain strains following the injection of large amounts of estrogens over extended periods of time (3).

The variations apparent in the responses of the mammary glands of different species of experimental animals enhances the desirability of conducting similar studies with primates. Allen (4) observed the partial restoration of the atrophic breasts of ovariectomized monkeys following the injection of estrin. Turner and Allen (5) observed a partial growth of the mammary ducts of male, and probably some alveolar development in the glands of ovariectomized female monkeys receiving estrogens. Aberle (6) obtained inconclusive or no evidence of mammary growth in young female monkeys following the injection of theelin for short periods. The growth of the mammary ducts was observed in young male monkeys following the injection of theelin for periods up to 42 days (7).

A study of the breasts of many monkeys, which have received estrogens over brief intervals, indicated that mammary growth may require a comparatively long time in this species. The present experiments have been conducted to determine critically the rate and nature of the mammary growth occurring in monkeys receiving estrogens. An extensive series of mammary glands from monkeys of known ages is being collected by one of us (G. v. W.) and it is probable that age, rather than weight, may soon be used in following the rate of normal breast development.

## MATERIALS AND METHODS

Sixteen male and 2 immature ovariectomized female monkeys (Macacus rhesus) were used in the present experiment. Body weights at the end of the

[1] This investigation has been aided by grants from the Committee for Research in Problems of Sex of the National Research Council, the Fluid Research Funds of Yale University School of Medicine, and the Anna Fuller Fund.

experiments ranged from 1830 to 7220 gm. for the males, and 3135 and 3440 gm. for the ovariectomized females. To indicate the original extent of growth of the mammary tissue, one breast was removed from each of 4 of the males and from one of the females prior to treatment. The glands removed surgically

TABLE 1. SUMMARY OF INJECTIONS AND BREAST CHANGES IN MALE MONKEYS RECEIVING DAILY INJECTIONS OF ESTROGENS FOR PERIODS OF 6 TO 42 DAYS

| Animal no. | Wt. at end of injections gm. | Units daily | No. days injected | Total units | Measurements[1] experimental mm. |
|---|---|---|---|---|---|
| 27 | 2400 | 20 I.U. theelin in oil | 6 | 1200 I.U. | *R.* 5.5× 5.0<br>*L.* 5.0× 4.5 |
| 25 | 3040 | 500 R.U. mare estrin | 12 | 6000 R.U. | *R.*10.0× 7.0 |
| 58 | 5735 | 250 R.U. theelin | 12 | 2750 R.U. | *R.*11.0×10.0 |
| 90 | 6555 | 500 I.U. theelin in oil | 12 | 6000 R.U. | *R.* 8.5× 9.0<br>*L.* 8.0× 8.5 |
| 30 | 1830 | 200 I.U. theelin in oil | 15 | 3000 I.U. | *R.* 8.0× 8.5<br>*L.* 9.3× 9.5 |
| 01 | 2580 | 200 I.U. theelin in oil | 15 | 3000 I.U. | *R.* 7.0× 4.5<br>*L.* 6.0× 4.0 |
| 99 | 2615 | 200 I.U. theelin in oil | 15 | 3000 I.U. | *R.* 4.5× 4.6 |
| 07 | 2915 | 200 I.U. theelin in oil | 15 | 3000 I.U. | *R.* 5.5× 5.5<br>*L.* 5.5× 5.0 |
| 06 | 3600 | 200 I.U. theelin in oil | 15 | 3000 I.U. | *R.*11.0×14.0 |
| 23 | 2620 | 200 I.U. theelin in oil | 16 | 3200 I.U. | *R.*23.0×21.0<br>*L.*19.0×17.5 |
| 16 | 3005 | 100 R.U. theelol | 20 | 2000 R.U. | *R.*26.5×22.0[2] |
| 03 | 7220 | 250 I.U. theelin in oil | 20 | 5000 I.U. | *L.* 2.5× 3.0[3] |
| 86 | 2450 | 100 R.U. theelin | 34 | 3000 R.U. | *R.* 7.0× 9.0<br>*L.* 6.5× 6.5 |
| 36 | 2000 | 200 R.U. theelin | 42 | 5000 R.U. | *R.*22.0×14.0<br>*L.*20.5×15.0 |

[1] All measurements are caudo-cephalic and ventro-lateral. *R* = right gland, *L* = left gland.
[2] Control mammary gland, *L*20.0 x 23.0 mm.
[3] Control mammary gland, *R*2.5 x 2.0 mm.

were flattened, fixed in Bouin's fluid and stained in borax carmine or Mayer's hemalum. Such preparations enable a reliable comparative estimation of the size of the glands and at the same time afford a microscopic study.

Theelin as a solution in oil or water, theelol[2], a preparation of estrogenic

[2] The theelin and theelol used in these experiments were generously supplied by Parke, Davis & Co., through the courtesy of Dr. Oliver Kamm.

hormones from the urine of pregnant mares[3], and hydroxyestrin benzoate[4] were the estrogens used. All of the injections were made subcutaneously or intramuscularly. Some of the monkeys received daily injections (table 1) and others received two injections per week, (table 2). The periods of treatment varied from 6 days to 22 weeks.

Measurements of the nipples of those animals on experiment over the longer periods were made at weekly intervals. The experimental glands removed at autopsy were fixed on the skin and then dissected, stained and studied as whole gland preparations. The nipples and small pieces of the glands were examined histologically. All of the whole glands were measured and the greatest caudo-cephalic and ventro-lateral dimensions were determined (tables 1 and 2).

TABLE 2. SUMMARY OF INJECTIONS AND BREAST CHANGES IN OVARIECTOMIZED FEMALE AND YOUNG MALE MONKEYS RECEIVING HYDROXYESTRIN BENZOATE

| Animal no. | Sex | Wt. at start gm. | Wt. at end gm. | Nipple Measurements | | | | I.U. Weekly | Weeks injected | Total I.U. | Mammary Gland Measurements | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Start | | End | | | | | Control gland mm. | Exper. gland mm. |
| | | | | H mm. | W mm. | H mm. | W mm. | | | | | |
| 70 | ♀ | 3320 | 3440 | 9.5 | 7.0 | 10.5 | 8.0 | 2000 | 9 | 18000 | | 71×52 |
| 74 | ♀ | 3220 | 3135 | 7.0 | 5.5 | 7.0 | 6.0 | 4000<br>8000 | 9<br>12.5 | 136000 | 35×26 | 61×47 |
| 71 | ♂ | 3545 | 3850 | 3.0 | 5.5 | 8.5 | 6.0 | 4000 | 9 | 36000 | 12× 8 | 33×24 |
| 72 | ♂ | 3165 | 3310 | 4.0 | 5.0 | 7.0 | 5.0 | 4000 | 22 | 88000 | 8× 8 | 54×48 |

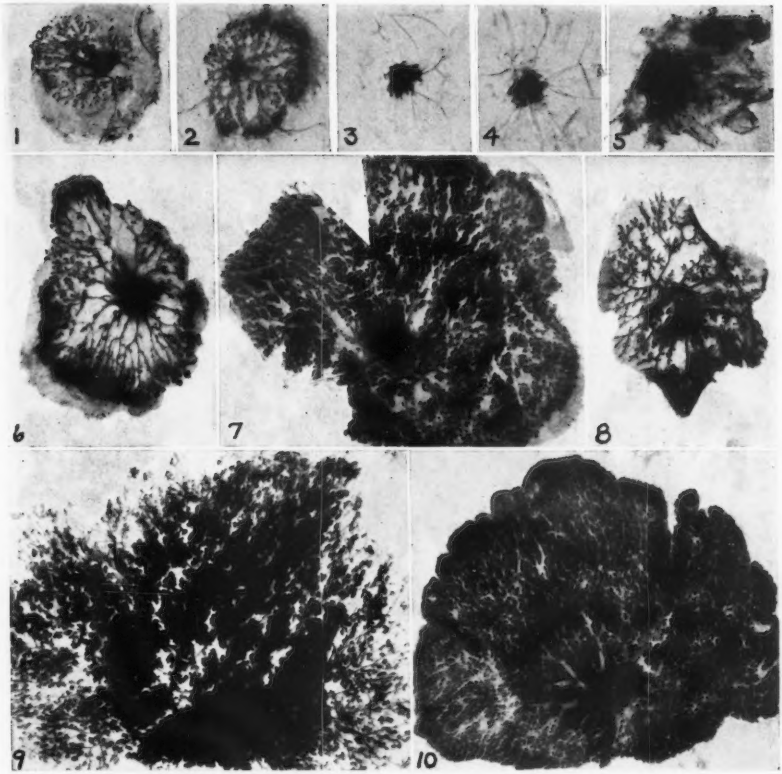
## OBSERVATIONS

*Size of the control glands.* The size of the control glands removed from the different male monkeys prior to the administration of estrogens varied greatly, the extremes being 2.5 x 2.0 mm. and 20.0 x 23.0 mm. Although the smallest control gland was removed from the largest monkey in the present series, there is no evidence from present observations that weight or age may have any uniform effect upon the size of the male breast. The normal development of the male breast will be described in detail in a later paper. The variations in size of the mammary glands of different animals is mentioned here only to emphasize the necessity of adequately controlling such experiments as those reported here.

*Size of the experimental glands.* The mammary glands of only 2 of the male monkeys receiving estrogens for 42 days or less exceeded in dimensions the control glands of one of the animals (*16*, table 1). A slightly larger right experimental breast was observed in this animal after it had received 2000 R.U. of theelol during 20 days (fig. 1 and 2). On the other hand, the experimental breast of another monkey (*03*) was insignificantly larger than the control gland, although it had received 5000 I.U. of theelin during 20 days.

[3] The estrogenic materials obtained from the urine of pregnant mares contain 2000 R.U. per cc. and were kindly supplied by Dr. H. H. Cole of Davis, California.

[4] The hydroxyestrin benzoate (oestradiol-benzoate) is commercially known as dimenformon, and was obtained in crystalline form from the Organon Laboratories. It was dissolved in oil, 1 mg. per cc.




Photographs of the dissected and stained mammary glands of male monkeys, Fig. 1-7. Mammary glands of immature, ovariectomized female monkeys before and after treatment with hydroxyestrin benzoate, Fig. 8-10.

Fig. 1. The left mammary gland of a male monkey (*16*) weighing 3005 gm. and removed prior to the administration of theelol. The gland measured 20 x 23 mm. and consisted of branching ducts. x¾.

Fig. 2. The right mammary gland of monkey *16* removed after the injection of 2000 R.U. of theelol in 20 days. This gland was definitely larger than the control breast. The laterally, inferiorly and superiorly directed ducts terminated in hyperchromatic buds in cellular stroma. These peripheral changes are early indications of rapid mammary growth. x¾.

Fig. 3, 4. The left and right mammary glands of a male monkey (*90*) weighing 6555 gm. removed after 6000 I.U. of theelin had been injected during 12 days. The glands consisted entirely of ducts and showed no evidence of recent growth. x¾.

Fig. 5. The left mammary gland of a male monkey (*71*) weighing 3545 gm., removed at operation. The gland consisted of thin primary ducts and a few short secondary branches which were confined to an area not exceeding 12 x 8 mm. x1.

Fig. 6. The right mammary gland of monkey *71* removed at autopsy after 36000 I.U. of hydroxyestrin benzoate had been injected subcutaneously or intramuscularly during 9 weeks. This gland was approximately 3 times the size of the control left breast, but still consisted only of a branching system of ducts. The peripheral growth of the gland was still occurring, as indicated by the slightly distended and hyperchromatic end buds and the increased number of connective cells in this region. x1.

Fig. 7. The right mammary gland of a male monkey (*72*) removed at autopsy after it had received 88000 I.U. of estrogen in 22 weeks. The left gland removed prior to treatment had not exceeded 8 x 8 mm., while the right gland measured 54 x 48 mm. There was some evidence that a peripheral growth was still in progress. The gland now consists of branching ducts and many completely formed and other partially formed lobules of alveoli. The ducts contained some fluid. A part of the gland was cut out for sectioning and microscopic examination. x1.

Fig. 8. The gland of monkey *74* removed at operation at the same time ovariectomy was performed. A small part of the gland was not obtained. The gland consisted of a branching system of ducts, and at the time of removal showed no evidence of rapid proliferation. The small piece of gland which was not removed grew during the following treatment. x1.

Fig. 9. The gland of monkey *70* removed at autopsy after 18,000 I.U. of hydroxyestrin benzoate had been injected during 9 weeks. Although a control gland was not removed from this animal it is certain that some growth had occurred. The initial body weight was very similar to that of monkey *74* above. There was little evidence of rapid peripheral growth. Complete or nearly complete lobules were present throughout the central part of the gland, while incomplete lobules and branching ducts predominated peripherally. x1.

Fig. 10. The right mammary gland of an ovariectomized female (*74*) monkey removed at autopsy after 136,000 I.U. of estrogen had been injected during 21.5 weeks. Although the gland was somewhat smaller than that of an adult female monkey, well-developed lobules were present throughout. Rapid peripheral growth was not apparent. The ducts and alveoli were slightly distended with secretion. Cystic terminal distension of the ducts occurred at one point along the periphery. x1.

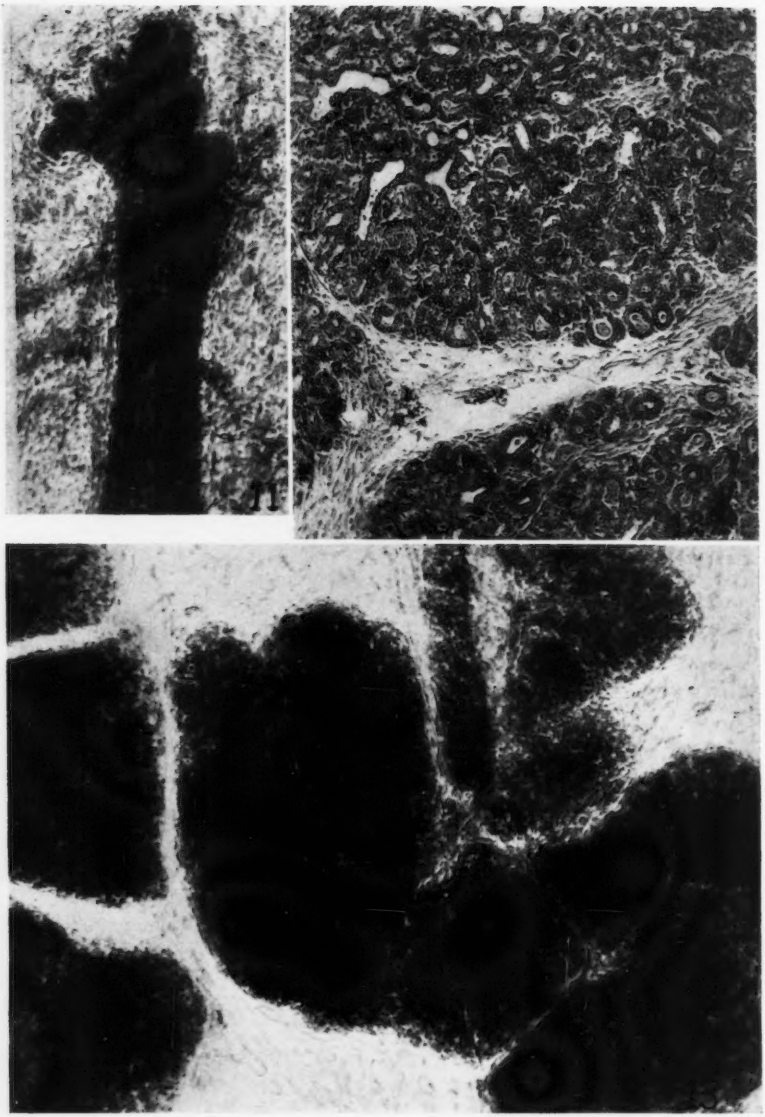


Fig. 11. The end of one of the primary ducts of the control mammary gland of a young male monkey (*72*) photographed from the gross mount of the gland. Structurally, the gland was similar to that of monkey *71* (fig. 5). x108.

Fig. 12. A photomicrograph of the breast of monkey *74* removed after 136,000 I.U. hydroxyestrin benzoate had been injected during 21.5 weeks. (See fig. 10 for the gross preparation of the gland of this monkey.) Well-developed lobules were present. Some of the alveoli contained secretion but the gland was not comparable to that of a lactating animal. x108.

Fig. 13. A photomicrograph of a portion of the gland shown in figure 7, indicating the extent to which histological details can be determined by microscopic examination of the whole gland preparations. x108.

The control glands of the 2 male monkeys which received weekly injections of 4000 I.U. of hydroxyestrin benzoate measured 12.0 x 8.0 mm. (fig. 5) and 8.0 x 8.0 mm., while the experimental glands removed 9 and 22 weeks later measured 23.0 x 24.0 mm. (fig. 6) and 54.0 x 48.0 mm. (fig. 7) respectively. The experimental glands had increased approximately 3 times in size during the 9-week period of injections, and approximately 6 times during the 22-week period (table 2).

A control gland was removed from one of the immature ovariectomized female monkeys (table 2). A small amount of mammary tissue in the upper lateral part of the gland was not obtained at the operation, and so the dimensions of the glands are given as 35.0 and 26.0 mm. (fig. 8). The experimental gland was slightly less than twice as large (61.0 x 47.0 mm.) (fig. 10) 21.5 weeks later, after 136,000 I.U. of hydroxyestrin benzoate had been injected.

As mammary glands were not removed from the other males or the other ovariectomized female, the estimations of size changes could not be made. This does not mean that growth had not occurred, as will be pointed out below. It does indicate that it is necessary to control the experiments in the individual animal, male as well as female, particularly in the shorter experiments.

*Growth of the nipples in monkeys receiving estrogens.* The nipples of the two immature ovariectomized female monkeys receiving estrone increased slightly in size during the course of the experiment (table 2). However, the nipples were partially developed at the beginning of the experiment. A definite growth of the nipples of the two males was observed during the 9 and 22-week periods in which estrogens were injected. During this interval, the small nipples of the males approximately doubled and tripled in length. It was also observed that nipples hypertrophied with relative speed after the injections were started, the nipple of the animal receiving estrogen for 9 weeks showing more enlargement than the monkey treated for a longer period. Measurements were taken at weekly intervals, and the size of the nipples in the other animals was found to be maximal at about this same time.

*Structural Changes in the Mammary Glands.* The mammary glands of the male monkeys removed surgically prior to the injection of estrogens varied greatly in structure as well as in size. All the glands consisted of more or less branched ducts. The smaller glands consisted largely of the primary ducts and very short secondary branches. The ducts of the larger glands were longer and more branched (fig. 1, 5). The terminal ends of the comparatively narrow branch and primary ducts were usually irregular (fig. 11). Lobules of alveoli were not observed in any of these glands.

The glands of only one of the male monkeys receiving estrogens for 15 days or less showed changes that might indicate active proliferation (monkey *58*). In this one animal there was a peripheral hyperchromatic area in the stained glands, a change that constituted one of the earliest indications of growth following the injection of estrogens. The terminal ends of the ducts were slightly distended and composed of irregularly arranged hyperchromatic cells. The surrounding connective tissue into which the buds were growing was more cellular than normal (fig. 2, 6). The ducts may be slightly larger, and

were occasionally filled with fluid. A marked increase in the number of small lateral branches was not apparent in the earlier stages. In the glands of all monkeys (with the exception of *03*) treated for 16 to 42 days evidence of peripheral growth was observed, although there was little (fig. 1, 2), or else an undetermined, increase in size.

A similar type of mammary gland was obtained from a male monkey which had received 36,000 I.U. of hydroxyestrin benzoate during a 9-week period (fig. 6). The glands consisted of branching ducts terminating at the periphery in the densely staining and cellular parenchymal and stromal tissue previously mentioned. During the first 2 months, the changes were limited essentially to an increase in the area occupied by the branching ducts.

The development of lobules was first observed in the gland of the male monkey which had received estrogen for 22 weeks. Well-developed lobules composed of well-formed alveoli were found throughout the greater part of the gland (fig. 7). At the periphery the lobules were not completely formed, and in places only branching ducts were present. The lobules as well as the glands had increased greatly in size. This monkey had received colchicine[5] prior to autopsy, and the accumulated mitotic figures showed that rapid growth was still in progress throughout the entire gland.

The control gland removed from the immature ovariectomized female monkey was about the same size as the gland of the male monkey which had received estrogens for 9 weeks. Structurally, the glands were similar except that the former showed no indication of peripheral hyperplasia.

Nine weeks after the injections of estrogens had been started, the gland of one of the ovariectomized female monkeys (*70*) contained a large central area of well-formed lobules (fig. 9). Peripherally, the lobules were less completely developed, in places only branching ducts having formed. Structurally, the gland somewhat resembled that of the male monkey which had received estrogens for 22 weeks although it was not quite as extensively developed.

The gland removed from the immature ovariectomized female monkey following the injection of 136,000 I.U. of estrogen during 21.5 weeks consisted of branching ducts obscured by the dense and numerous lobules (fig. 10, 12, 13). The larger ducts were distended with a slightly opaque fluid. Several drops of watery fluid had been removed at weekly intervals during the last two months when estrogen was injected. Except for its somewhat smaller size, the gland resembles those of some adult female monkeys or of pregnant animals.

## DISCUSSION

As this subject has been recently reviewed (1, 2), an extensive discussion of species variations in the response of the mammary glands to estrogens is not warranted. A large number of monkey mammary glands have been studied in connection with other experiments, but adequate control tissue had not been obtained, nor have sufficiently extended periods of treatment been undertaken to determine definitely the influence of estrogens.

Compared to the other laboratory animals that have been studied with

[5] Colchicine has recently been extensively used in determining the intensity of growth of genital tissues of mice. Recent papers and the earlier investigations are cited by Allen, Smith and Gardner (14).

respect to mammary development, the monkey develops and matures slowly. The breasts of normal young females increase slowly in size during many months (unpublished data, 8) and usually consist of a branching duct system which may or may not (fig. 8) be undergoing rapid proliferation. Even in the immature female monkey the breasts may proliferate periodically.

When an apparent excess of estrogens is administered (determined by pronounced sex skin swelling, circumanal swelling in males and the uterine picture in females), the glands of even experimental animals undergo a comparatively prolonged period of development. One breast of the male monkey receiving estrogen for 22 weeks was as yet incompletely developed, while that of the ovariectomized female, the control gland of which was about the size of that of the male following 9 weeks of estrogen injection, was completely developed 21.5 weeks later. It might be assumed that at least 30 weeks would be required for complete mammary development in the monkey under experimental conditions.

The glands of the monkeys receiving estrogen over the longer periods were not so large as those of adult female monkeys weighing several kg. more (unpublished data). Structurally, they resembled the glands of monkeys in which lactation has been induced experimentally (9). Although the gland of one monkey was structurally complete, its gross size was apparently in proportion to the size of the bearer. Aberle (8) observed an increase in the glands of female monkeys until a weight of 5000 gm. was attained.

The initial changes occurring in animals receiving estrogens were largely confined to a proliferation of the branching ducts and an enlargement of the breast area by peripheral growth (7). In most instances this growth is greatest at the lateral, inferior and superior borders. Later the peripheral growth of the ducts and the formation of lobules of alveoli occur simultaneously, the latter progressing from the region of the nipple to the periphery.

Whether the injection of larger or smaller amounts of estrogen would be followed by differences in the rates or types of mammary growth cannot be determined from present experiments. The different estrogenic chemicals, theelin, theelol and hydroxyestrin benzoate, were followed by similar mammary changes as far as could be determined under the conditions of the present experiments. In mice, it is quite obvious that variation in the amount of estrogens may influence particularly the type of growth (3), although following very large doses of estrogen the mammary glands of male rabbits consist only of branching ducts (unpublished data) as described by Turner and Frank (10).

Likewise in the above experiments the influence of progesterone has not been determined. It is possible that the corpus luteum hormone may augment or alter the effects of estrogens, or that it might independently stimulate breast hyperplasia, as shown in the mouse (11).

Although the nipples of only 4 animals were measured, it seems that nipple growth would not afford much information concerning the extent of mammary development, as was observed in the guinea pig (12, 13). The weekly measurements of nipples showed comparatively early development, and remained quite unchanged during the latter part of the experiment.

SUMMARY

The development of the mammary glands of male or ovariectomized immature female monkeys (Macacus rhesus) follows the administration of estrogenic hormones (theelin, theelol and hydroxyestrin benzoate). The glands first grow peripherally, and later lobules develop, appearing first near the nipple. Under the conditions of the present experiments it was found that more than 22 weeks are required for the mammary glands of young male monkeys to develop completely, and 30 weeks are indicated for complete development.

The growth of the nipple does not afford an index of the development of the breast tissue. The nipples reach their maximal proportions after 8 to 10 weeks of treatment.

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# QUANTITATIVE CYTOLOGICAL STUDIES OF THE ANTERIOR LOBE OF THE HYPOPHYSIS OF FETUSES AND CHILDREN, CORRELATED WITH SEXUAL AND SKELETAL DEVELOPMENT[1]

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The increasing interest in the hypophysis cerebri has resulted in a demand for a more accurate knowledge of the cytology of this gland during fetal life and infancy. In the White House Conference Report on the Growth and Development of the Child (1) Hoskins states in this connection "that many more autopsy studies, with special attention to cytological technique are needed." In making quantitative cytological studies on normal adult male and female pituitaries, Rasmussen (2, 3) noted a significant difference between the sexes, and concluded that "the next step would be to see at what age period this sex difference arises." The present study was undertaken with this purpose in mind. In addition to establishing a norm, an attempt has been made to correlate the relative percentages of the three cell types with sex, body length, and body weight.

There are isolated references in the literature to the fetal and infant pituitary, but to our knowledge no quantitative study of this problem has ever been made. Cooper (4) has reviewed the literature and made a detailed study of the pituitaries of the fetus and child. According to her, the first cells to appear are the undifferentiated ones, or chromophobes. By the third fetal month the granular cells appear. At this time acidophiles are seen, and by 3½ months, basophiles have made their appearance. This is contrary to the observations of Fraser (5), who stated that the basophiles appeared first. Cooper considers the neutrophile as a distinct cell, and differentiates it from the chromophobe or undifferentiated cell. She first noted the appearance of neutrophiles in a 7-month fetus. At the present time, most writers include the small cell with scanty cytoplasm, and the larger cell with neutrophilic cytoplasm, as chromophobes. In this study, the term chromophobe will include both cell types. Cooper estimates that during intra-uterine life the undifferentiated cells are in the majority, the acidophiles are the most numerous of the granular cells, the neutrophiles next, and the basophiles least of all. During childhood the undifferentiated cells have been replaced by the granular cells, with the acidophiles in the majority, the neutrophiles next in number, and basophiles least in number.

## MATERIAL AND METHODS

The technique established by Rasmussen (2, 3, 6) in studying the normal adult gland was rigidly adhered to. Briefly, this consists of Zenker fixation,

[1] A preliminary report of this work was awarded the Phi Delta Epsilon Research Prize and published in the Phi Delta Epsilon News of March, 1937.

serial sectioning of paraffin blocks in a horizontal plane at 5 μ, and mounting sections from the upper, middle and lower thirds of the gland. Various stains were tried, but the simplest and most consistent was a modified Martins' (7) stain. As modified by us for use on human material, this method consists in first staining for 30 seconds in a 0.5 per cent aqueous solution of erythrosin, draining off the excess stain, and passing directly into a 1 per cent aqueous solution of phosphomolybdic acid for 90 seconds, draining, and passing directly into a 0.5 per cent aqueous solution of methyl blue for 50 to 60 seconds, differentiating in 95 per cent alcohol, and then passing through absolute alcohol and xylol to mount in balsam. The amount of time given is average, and may have to be varied slightly. Preliminary staining in hematoxylin to bring out nuclear detail may occasionally be necessary, but as a rule methyl blue stains the nucleus well. The best section mounted from each of the 3 levels was studied under oil immersion. All the cells in every fifth field were counted and the results statistically analyzed. The average number of cells in a field is 100, and as 20 to 75 fields were examined the total number of cells counted in each pituitary ranged from 2,000 to 7,500. According to Rasmussen, the percentages of the 3 cell types obtained by this method are within an error of 2 per cent.

One hundred and three glands were studied. There were 54 males and 33 females less than 55 cm. in length, and 9 males and 7 females greater than 55 cm. in length. Since 55 cm. is the greatest crown-heel length attained by any new-born in this series, it is used as an arbitrary line of division to separate fetuses and stillborns from those cases having a significant postnatal existence. The latter represent a heterogenous group that lived from a few months to 14 years. In the case of fetuses and stillborns, death was 'accidental', i.e., reported by the pathologist as due to cerebral hemorrhage, atelectasis, prematurity, etc. In the older children, death was often due to one of the common childhood diseases. No gland was used which the pathologist considered abnormal or in which a good differential stain could not be obtained.

## OBSERVATIONS

*Frequency distribution.* The distribution of the percentages of the 3 cell types is shown in the charts in figures 1 and 2. The distribution of the basophiles in the group of males less than 55 cm. is skewed in a positive direction, similar to the results published by Rasmussen for the adult. The other cell types tend to form a more symmetrical curve in the frequency distribution charts.

The common statistical methods were used to derive the median, mean or average, probable error, standard deviation, and coefficient of variation. One probable error includes 50 per cent of the cases, and two probable errors include 95 per cent. Probable error rather than standard error was used because it is more familiar to most scientific investigators.

*Percentages of the 3 cell types in the male, table 1.* The chromophobes have a coefficient of variation between 17 and 19.5, which is almost the same

amount of variability as the weight of the normal adult heart, whose coefficient of variation is 18. The acidophiles and basophiles from glands of fetuses and stillborns less than 55 cm. in length have coefficients of variation of 40 and 48.7, respectively, which are nearly of the same variability as the normal thyroid, whose coefficient of variation is 45. The acidophiles and

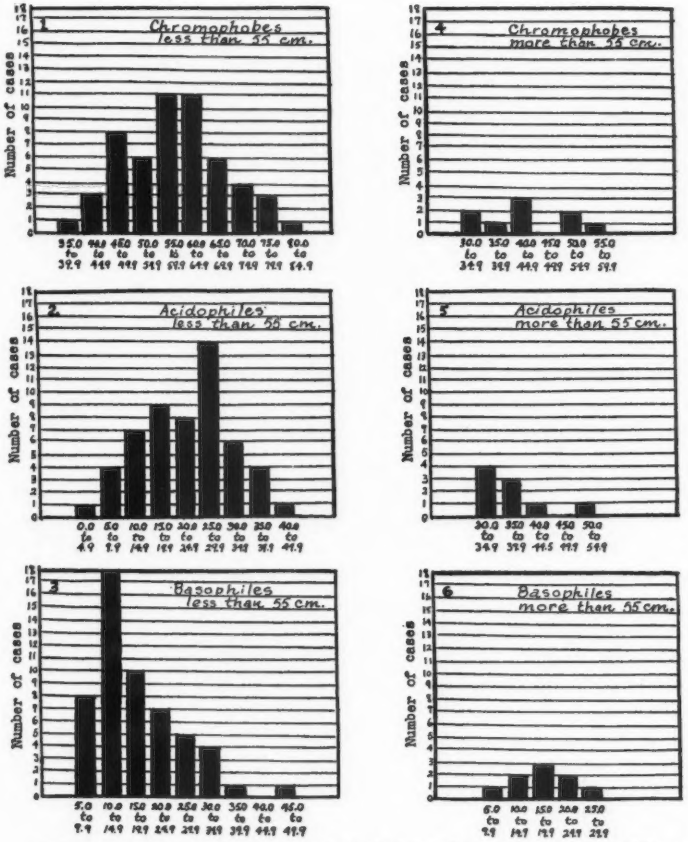
1 Chromophobes less than 55 cm.
2 Acidophiles less than 55 cm.
3 Basophiles less than 55 cm.
4 Chromophobes more than 55 cm.
5 Acidophiles more than 55 cm.
6 Basophiles more than 55 cm.
Number of cases

Fig. 1. Charts 1 to 6, showing frequency distributions of the percentage of the different types of cells in the anterior lobe of the hypophysis of males of 2 groups, one of cases less than, and the other of cases greater than, 55 cm. in length.

basophiles in the glands from the older cases have a similar coefficient of variation.

*Percentages of the 3 cell types in the female, table 2.* In the hypophyses from cases less than 55 cm. in length, the coefficients of variation of the 3 cell types are very similar to those noted in the males of the younger series. The chromophobes, basophiles and acidophiles from the older series have in each instance a smaller degree of variability than the same cell type of the younger cases.

In table 3 the differences in the average percentages of the 3 cell types were determined according to sex. Since the number of cases greater than 55 cm. in length was too small to analyze statistically, only males and females under 55 cm. in length were compared with each other.

Table 4 was constructed to observe if, among cases less than 55 cm. in length, any correlation existed between the percentages of the different cell

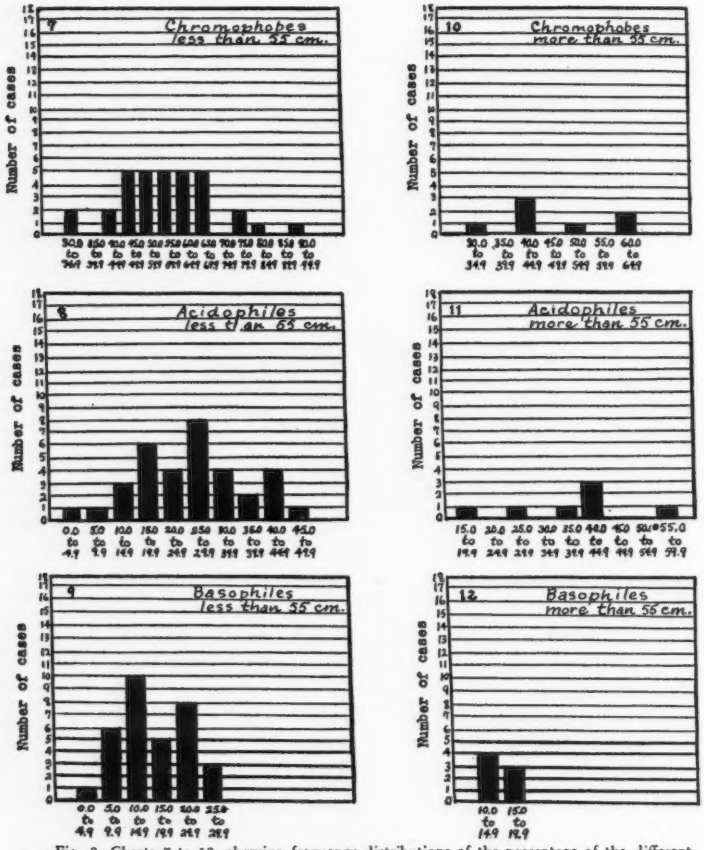
7 Chromophobes less than 55 cm.
10 Chromophobes more than 55 cm.
8 Acidophiles less than 55 cm.
11 Acidophiles more than 55 cm.
9 Basophiles less than 55 cm.
12 Basophiles more than 55 cm.
Number of cases

Fig. 2. Charts 7 to 12, showing frequency distributions of the percentage of the different types of cells in the anterior lobe of the hypophysis of females of 2 groups, one of cases less than, and the other of cases greater than, 55 cm. in length.

types and skeletal development as represented by body length and body weight.

Our technic is similar to Rasmussen's in almost every detail. It does differ in that we used Zenker's fixer instead of formalin, and a differential stain which is a variation of the Mallory connective tissue stain as used by him. The average percentages of the 3 cell types obtained by Rasmussen in adults of both sexes were compared with those found by us in cases less than 55 cm.

in length. In each instance and in the case of both sexes, a significant statistical difference was noted. The chromophobes and basophiles were more numerous, and the acidophiles less numerous in the fetus than in the adult.

## DISCUSSION

The average, or mean, percentages of the 3 cell types, both in males and females, have a fairly constant coefficient of variation. In only one instance

TABLE 1. PERCENTAGES OF THE THREE CELL TYPES IN THE ANTERIOR LOBE OF THE MALE HYPOPHYSIS.

| Cell Type | Length | Minimum | Maximum | Median | Mean and P.E. of *M* | Std. Dev. ± | Coefficient of Variation % |
|---|---|---|---|---|---|---|---|
| Chromophobes | Less than 55 cm. | 35.3 | 81.5 | 59.1 | 59.1±0.93 | 10.1 | 17.0 |
| | More than 55 cm. | 33.3 | 57.7 | 43.3 | 43.6±2.03 | 8.5 | 19.5 |
| Acidophiles | Less than 55 cm. | 2.5 | 40.0 | 23.8 | 22.8±0.84 | 9.1 | 40.0 |
| | More than 55 cm. | 30.6 | 51.4 | 36.7 | 37.5±1.49 | 6.3 | 16.7 |
| Basophiles | Less than 55 cm. | 6.1 | 45.7 | 15.5 | 18.0±0.81 | 8.8 | 48.7 |
| | More than 55 cm. | 9.7 | 27.9 | 18.3 | 17.5±1.37 | 5.8 | 32.9 |

(basophiles in males less than 55 cm. in length) does the average percentage of a cell type have a greater amount of variability than the weight of the normal human thyroid. The chromophobes are the least variable, while the acidophiles and basophiles have a larger coefficient of variation. This is similar to Rasmussen's observations in the adult (2, 3).

Our results are not in complete agreement with the estimates of Cooper (4)

TABLE 2. PERCENTAGES OF THE THREE CELL TYPES IN THE ANTERIOR LOBE OF THE FEMALE HYPOPHYSIS.

| Cell type | Length | Minimum | Maximum | Median | Mean and P.E. of *M* | Std. Dev. ± | Coefficient of Variation % |
|---|---|---|---|---|---|---|---|
| Chromophobes | Less than 55 cm. | 31.1 | 90.2 | 58.0 | 58.1±1.54 | 12.9 | 22.2 |
| | More than 55 cm. | 33.3 | 64.3 | 45.0 | 48.2±2.85 | 10.4 | 21.5 |
| Acidophiles | Less than 55 cm. | 3.0 | 47.5 | 26.8 | 26.6±1.24 | 10.4 | 39.1 |
| | More than 55 cm. | 19.6 | 55.2 | 41.7 | 38.2±3.25 | 11.8 | 30.9 |
| Basophiles | Less than 55 cm. | 4.8 | 26.5 | 15.0 | 15.8±0.80 | 6.7 | 42.3 |
| | More than 55 cm. | 11.0 | 16.4 | 15.0 | 14.6±0.68 | 2.48 | 16.9 |

on the respective numbers of each of the cell types. As Cooper stated, during intra-uterine life, the chromophobes or undifferentiated cells are in the majority, but contrary to her findings the acidophiles in many glands are outnumbered by the basophiles. In the child, Cooper estimated that the acidophiles were most numerous, that the neutrophiles came next, and that the basophiles were least numerous. This is not in accord with the quantitative

studies of Rasmussen on the adult, or our findings in the child. As Rasmussen pointed out, the large brilliantly stained acidophiles overshadow the small chromophobes, which often have only a nucleus, with little or no cytoplasm. Therefore, estimates cannot be an accurate index of the true state of the gland.

A comparison of the average percentages of the different cells in glands, between cases less than 55 cm. in length and those greater than 55 cm. in length indicates that the chromophobes decrease as the child grows older,

TABLE 3. COMPARISON OF THE MEAN PERCENTAGES OF THE THREE CELL TYPES IN THE HYPOPHYSIS OF MALES AND FEMALES LESS THAN 55 CM. IN LENGTH.

| Cell type | Males (54 cases) | Females (33 cases) | Difference | P.E. of difference | Difference / P.E. of difference |
|---|---|---|---|---|---|
| Chromophobes | 59.1±0.93 | 58.1±1.54 | 0.96 | 1.76 | 0.55 |
| Acidophiles | 22.8±0.84 | 26.6±1.24 | 3.81 | 1.49 | 2.6 |
| Basophiles | 18.0±0.81 | 15.8±0.80 | 2.13 | 1.13 | 1.88 |

while the acidophiles become more numerous. The basophiles show only a slight tendency to decrease with age. However, because the series above 55 cm. contained only 9 males and 7 females, it was not considered justifiable to include these comparisons, although tables were constructed in which these facts were substantiated by statistical analysis.

Rasmussen (2, 3) observed in adults that the female hypophysis had more acidophiles and fewer basophiles and chromophobes than the male. In our series, the only significant sex difference was a greater number of acidophiles

TABLE 4. COEFFICIENT OF CORRELATION ($r$) BETWEEN THE MEAN PERCENTAGES OF THE 3 CELL TYPES IN THE HYPOPHYSIS OF MALES AND FEMALES LESS THAN 55 CM. IN LENGTH, AND LENGTH AND WEIGHT.

| Cell type | $r$, Length | | $r$, Weight | |
|---|---|---|---|---|
| | Males | Females | Males | Females |
| Chromophobes | −0.60 ±0.059 | −0.69±0.063 | −0.49±0.074 | −0.55±0.09 |
| Acidophiles | 0.62 ±0.058 | 0.60±0.121 | 0.67±0.054 | 0.64±0.077 |
| Basophiles | 0.013±0.094 | 0.35±0.106 | −0.07±0.969 | 0.47±0.100 |

present in the female. It is impossible at this time to answer Rasmussen's question as to when the sex differences noted in the proportionate distribution of the 3 cell types in the adult are established. As has already been noted, the acidophiles were more numerous in females than in males, and therefore the sex difference in the case of this cell type must become apparent early in fetal life. Thus, we must conclude that the attainment of adult numerical proportions and the establishment of sex differences in distribution occurs later in the case of chromophobes and basophiles than in the case of acidophiles. Only by a study of more of the older children can we expect to learn when the baso-

philes and chromophobes become more numerous in the males than in the females, and when the adult numerical proportions are reached in both sexes. It seems probable that this occurs early in childhood, because Rasmussen's series, which began at 16 years of age, had already attained the adult type of cell distribution.

There is a definite correlation between the number of acidophiles present in a gland and the length and weight of the fetus. This is true only in the instance of cases less than 55 cm. in length. In the glands from cases greater than 55 cm. in length, inspection of scatter curves demonstrated that no such correlation existed. Rasmussen was also unable to find any correlation between the number of acidophiles and skeletal development. Weight follows length very closely, but due to such extraneous factors as nutrition, dehydration, etc., weight is a more variable factor than length. As the fetus increases in length and weight, the acidophiles and, to a much less extent, the basophiles increase in number, while the chromophobes decrease. This relationship between the number of the different cell types and length and weight apparently ceases to hold true soon after the birth of the child.

## SUMMARY

In 103 human fetuses and infants, the cells of the anterior lobe of the hypophysis were studied by counting with the oil immersion lens all the cells in every fifth field, from 3 different levels of the gland. According to the size of the gland, approximately 2,000 to 7,500 cells were counted in each case. The results obtained were statistically analyzed.

The cases studied were divided into 2 groups, one comprising those less than 55 cm. in length, the other those greater than 55 cm. in length. Fifty-five cm. represents the greatest length attained by any new-born in our series, while the cases above this length lived for varying lengths of time.

In males and females less than 55 cm. in length, the average percentage of each of the 3 cell types was as follows: males—chromophobes 59.1, acidophiles 22.8, and basophiles 18.0; females—chromophobes 58.1, acidophiles 26.6, and basophiles 15.8. In cases greater than 55 cm. in length, the results were as follows: males—chromophobes 43.6, acidophiles 37.5, and basophiles 17.5; females—chromophobes 48.2, acidophiles 38.2, and basophiles 14.6.

Females have more acidophiles than males of a similar group. Since this has also been observed by Rasmussen in adults, it appears as a definite sex difference which must be established early in fetal life. As described by Rasmussen, the chromophobes and basophiles apparently assume the adult type of distribution, with its sex differences, some time after birth but before puberty.

There is also a significant difference in the average percentages of the 3 different kinds of cells obtained by us in cases less than 55 cm. in length, and those found by Rasmussen in the adult. In the fetus, there are more chromophobes and basophiles and fewer acidophiles than in the adult.

A very definite correlation exists between length and weight and the num-

ber of acidophiles present. With an increase in length and weight there is a marked increase in the number of acidophiles, a slight increase in basophiles, and a decrease in chomophobes.

I wish to express my appreciation to Dr. F. B. Queen and Dr. M. C. Benner of the Department of Pathology for their cooperation in securing the hypophyseal glands used in this study.

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# THE COMPARATIVE EFFICACY OF VARIOUS ANDROGENS AS DETERMINED BY THE RAT ASSAY METHOD[1, 2]

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In the course of the chemical characterization of androsterone, one of the androgens isolated from male urine, Butenandt (1) found that the acetate prolonged the effect on the capon's comb. The oxime had a similar effect but was rather irregular in its action. Laqueur and his associates (2) found that testosterone, the crystalline androgen isolated from bulls' testes, did not have so great an effect on the accessory sex glands of castrate rats as slightly impure extracts. Its activity could be increased greatly by the addition of an 'x-substance,' obtainable from testes extracts, which was shown later to be probably a fatty acid (3, 4).

The present investigation was prompted by a desire to obtain more information concerning androgenic substances, with a view to selecting a product which would most nearly simulate the natural processes without too frequent injections. Such a product would greatly facilitate the clinical usage of androgenic materials.

## MATERIALS

Androstanediol benzoate, testosterone benzoate, androstenedione, testosterone oxime, testosterone, testosterone diacetate and testosterone propionate were provided by the Schering Corporation through the courtesy of Dr. E. Schwenk and Dr. G. Stragnell. The testosterone acetate was provided by the Ciba Company through the courtesy of Dr. C. C. Haskell. The urine extract was prepared according to the method used in our laboratory (5).

All the androgens were dissolved in the same lot of olive oil in such concentration that each injected dose would be contained in 0.3 cc. of the solvent. Other investigators (6, 7, 8, 9, 10) in the meantime have demonstrated that the amount and nature of the oil administered has a definite effect on the activity of certain androgens in the rat test.

## METHODS

Since the purpose of this study was to obtain data for clinical use, a mammalian test animal was chosen. The method of assay is similar in general to that employed by the various workers in this field (6, 9, 10, 11, 12, 13, 14). Care was taken to keep conditions as rigidly standardized as possible in every

[1] This investigation was aided by grants from the Committee on Scientific Research of the American Medical Association and the International Cancer Research Foundation.
[2] Most of the data contained in this paper were presented on April 23, 1937, before the American Physiological Society at the Federation of American Societies for Experimental Biology meeting at Memphis, Tenn.

respect. Only white rats from our colony were used. The animals were castrated at 70 to 90 gr. body weight, and were injected 28 to 32 days later. One day after the last injection, the rats were killed by a blow on the head, and the accessory sex glands removed and fixed in 10 per cent formalin for 24 hours. They were then transferred to 70 per cent ethyl alcohol, cleaned of extraneous tissue and, after drying between paper towels, weighed separately on a torsion balance.

Control rats gave average values of 6 mg. for the seminal vesicles and 35 mg. for the prostate. Since the time of castration influences the size of the organs and their response to androgenic materials (9), it is important that this condition be quite uniform for comparable results. For convenience of dissecting and weighing, the weights of the seminal vesicles include those parts of the prostate which run along the length of the seminal vesicles. These are

TABLE 1. THE EFFECT OF DOSAGE AND INTERVAL OF INJECTION ON THE REGENERATION OF THE PROSTATE AND SEMINAL VESICLES OF CASTRATE RATS BY TESTOSTERONE OVER A 6-DAY PERIOD

| Interval between Injections hours | Total dose mg. | No. of Rats | Wt. Increase | | Prost./Sem. Ves. Ratio |
|---|---|---|---|---|---|
| | | | Prostate[1] | Sem. Ves.[2] | |
| 48 | 0.72 | 6 | 5 | 3 | 1.67 |
| 24 | 1.44 | 6 | 25 | 19 | 1.32 |
| 48 | 1.44 | 6 | 12 | 8 | 1.50 |
| 24 | 2.88 | 6 | 50 | 32 | 1.56 |
| 48 | 7.20 | 6 | 25 | 16 | 1.56 |
| 24 | 7.20 | 3 | 87 | 54 | 1.61 |
| 24 | 14.40 | 6 | 114 | 69 | 1.65 |

[1] Controls, 35 mg.
[2] Controls, 6 mg.

termed the cranial lobes by Moore, Price and Gallagher (16). The weight of the prostate includes all the other lobes: *a)* the anterior lobes of Walker (15) which are termed the middle lobes by Moore, Price and Gallagher (16), and the dorso lateral lobes by Callow and Deanesly (14), and *b)* the ventral (14) or posterior (16) lobes and the anterior lobes (15). These are attached to the caudal end of the prostate only by their ducts, and care must be taken during dissection, especially in the smaller organs, not to remove them as extraneous tissue.

The standard procedure for assay at present employed in this laboratory consists of injections on 6 consecutive days, followed by removal of the accessory sex glands on the seventh day. Variations in this method of assay were made to suit the purposes of the experiments.

## RESULTS

*Testosterone.* At the time this study was begun, testosterone was the most active natural or synthetic androgen known. The results (table 1, fig. 1), with one exception, were obtained on a lot of rats all castrated at the same time, 6 rats being used for each determination. The values for the determination at a total dose of 7.20 mg. were obtained later, and only 3 rats were used. The smoothness of the curve is attributed in large part to the fact that the

conditions were so rigidly controlled. Subsequent assays of testosterone solutions have given values which could not always be placed at their proper places on the curves. This is not surprising when we consider that the amount of free fatty acids (2, 3, 6, 8) in the oil has a marked effect in the degree of activity of testosterone.

The solid lines in the graph show a rapid regeneration of both seminal vesicles and prostate, with increase in dosage followed by a tapering off, which

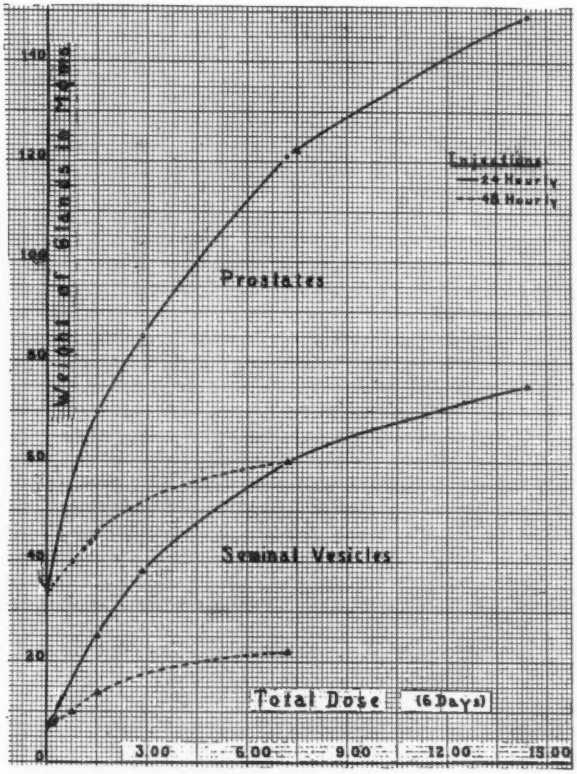


Fig. 1. Effect of dose and interval of injection on the regeneration of the prostate and seminal vesicles of castrate rats over a 6-day period. All of the values except those of the 24-hour injections at a total dose of 7.20 mg. were obtained simultaneously.

suggests a logarithmic relationship between dose and regeneration. The curves at the highest values obtained have already begun to flatten out. For example, a comparison of the value for the seminal vesicles at this point, 85 mg., with that in a normal rat of the same weight, 700-1200 mg., indicates that testosterone administered daily over a period of 6 days, even in extremely large doses, will never be able to regenerate the atrophied sex glands of the castrate rat to normal. However, this does not mean that the accessory sex glands, as will be

seen later, cannot be brought to normal size. The broken curves depict the values obtained by doubling the interval between injections. There is not only a decreased efficacy in this case, but also an earlier maximum effect which is much less than that obtained in the 24-hour injection experiments.

*Interval of injections and efficacy of testosterone in long-period experiments.* The marked decrease in efficacy and maximum action obtained by doubling the interval of injections in the 6-day experiment made it desirable to confirm this effect for experiments covering longer periods of time. In figure 2 is the graphical presentation of results obtained simultaneously on 3 groups of rats of 6 each. The first group received daily, except on the 6th and 13th day, 0.24 mg. of testosterone; the second group received the same treatment

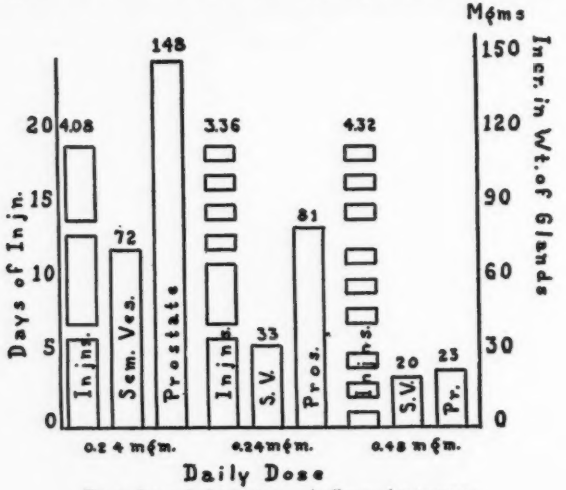


Fig. 2. Interval of injections and efficacy of testosterone in long-period experiments (19 days).

until the 12th day, when injections were made on alternate days (a total of 3 injections were omitted); the third group received on alternate days a double dose, 0.48 mg. The omission of the 3 injections in group 2 over the last half of the experiment approximately halved the response of the seminal vesicles and prostate. The doubling of the interval of injection was even more striking. In spite of the fact that the dose was doubled and the total dose was a little greater, the seminal vesicles are regenerated only approximately one-third and the prostate one-sixth that of the group receiving daily injections.

*Shortening of interval of injections and the efficacy of testosterone.* Since the efficacy of testosterone could be markedly diminished by lengthening the interval between injections, it seemed conversely, that it could be increased by shortening this interval. In figure 3 are given the results obtained by injecting the same testosterone preparation in 3 groups of rats (5 in each group) at 24-hour (1 x 0.3), 12-hour (2 x 0.15) and 8-hour (3 x 0.1) intervals. The increased efficacy is very striking, and occurs for both the seminal vesicles and prostate in a linear fashion.

Although now the efficacy of testosterone could be greatly enhanced by shortening the interval between injections, this method was entirely too impractical for clinical use. However, if this enhancing effect, obtained by mechanical means, could be obtained by some chemical alteration of the testosterone molecule, there would be available a convenient product for clinical administration.

*Comparative efficacy of various androgens.* Since Butenandt (1) had already demonstrated that esterification and oximation prolonged the effect of androsterone on the capon's comb, it seemed advisable to study similar alterations in the testosterone molecule. In addition, other androgens were available and also assayed according to our standard method for comparison with testos-

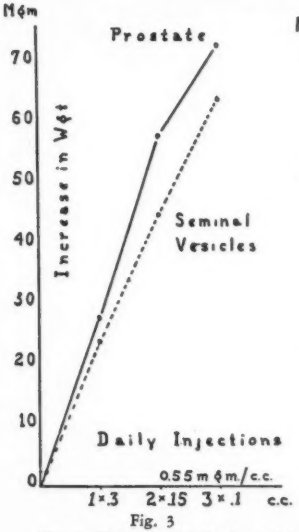


Fig. 3

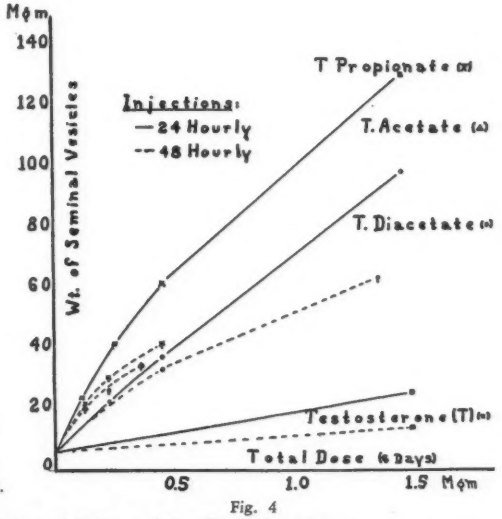


Fig. 4

Fig. 3. Shortening of the interval between injections and the efficacy of testosterone.
Fig. 4. The interval between injections and the efficacy of testosterone propionate, acetate and diacetate and testosterone.

terone. The tests were performed on 2 batches of rats 1 week apart and, therefore all the experiments may be considered as occurring simultaneously. Since the values (table 2) for the seminal vesicles show the same relative effects as for the prostates, only the latter are given in the graph (fig. 5).

Values along the abscissa for the urine extracts are in terms of liters. Assay of the extract by the capon method of Gallagher and Koch (17) indicated that 1 liter of urine contained 33 per cent more activity than 1 mg. of testosterone. Here the surprising fact is that urine extract, which has been demonstrated to contain as active material androsterone (18) and dehydroandrosterone (19), attains values in the rat test higher than that of testosterone. Experiments (9, 20) indicate that neither crystalline androsterone nor dehydroandrosterone is capable of attaining values anywhere nearly approaching that of testosterone in the rat test. Somewhat similar but not so striking an effect was noted by Callow and Deanesly (14). Two of their urine preparations had much the same effect

as crystalline androsterone, whereas a third extract was more than 3 to 4 times as great. Comparison of our results, capon unit for capon unit, indicates that our urine extracts are more effective in the prostate test than testosterone, which in turn is 6 to 10 times more active than androsterone (7, 19).

TABLE 2

| Androgen | Interval between Injections hr. | Total dose mg. | No. of rats | Wt. of Adrenals mg. | Wt. Increase | | Ratio of Prost./ Sem. Ves. |
|---|---|---|---|---|---|---|---|
| | | | | | Prostate mg. | Sem. Ves. mg. | |
| Urine Extract | 24 | 2.40[1] | 3 | 21 | 82 | 30 | 2.73 |
| | 24 | 4.50[1] | 3 | 22 | 109 | 47 | 2.32 |
| | 24 | 6.00[1] | 3 | 22 | 107 | 59 | 1.81 |
| Testosterone Benzoate | 24 | 2.00 | 2 | 22 | 27 | 13 | 2.08 |
| Androstanediol Benzoate | 24 | 1.50 | 3 | 19 | 2 | 3 | 0.67 |
| | 24 | 4.50 | 3 | 26 | 21 | 5 | 4.20 |
| Androstenedione | 24 | 1.50 | 3 | 20 | 19 | 7 | 2.71 |
| Testosterone Oxime | 24 | 1.50 | 4 | 21 | 23 | 11 | 2.09 |
| | 24 | 3.00 | 3 | 23 | 17 | 8 | 2.13 |
| | 24 | 4.50 | 3 | 25 | 26 | 16 | 1.63 |
| | 48 | 2.25 | 3 | — | 7 | 6 | 1.17 |
| Testosterone Propionate | 24 | 0.113 | 3 | 28 | 23 | 18 | 1.28 |
| | 24 | 0.25 | 3 | 23 | 42 | 36 | 1.17 |
| | 24 | 0.45 | 3 | 23 | 84 | 56 | 1.50 |
| | 24 | 1.50 | 4 | 29 | 156 | 125 | 1.25 |
| | 48 | 0.113 | 3 | — | 16 | 23 | 1.44 |
| | 48 | 0.225 | 3 | 22 | 25 | 36 | 1.44 |
| | 48 | 0.45 | 3 | — | 36 | 48 | 1.34 |
| Testosterone Acetate | 24 | 0.24 | 3 | 27 | 68 | 46 | 1.48 |
| | 24 | 0.72 | 3 | 31 | 115 | 68 | 1.69 |
| | 24 | 1.44 | 3 | 21 | 155 | 120 | 1.29 |
| | 48 | 0.12 | 3 | — | 24 | 15 | 1.60 |
| | 48 | 0.36 | 3 | — | 40 | 29 | 1.38 |
| Testosterone Diacetate | 24 | 0.113 | 3 | 22 | 8 | 6 | 1.33 |
| | 24 | 0.225 | 3 | 27 | 17 | 17 | 1.00 |
| | 24 | 0.450 | 3 | 26 | 49 | 32 | 1.53 |
| | 24 | 1.440 | 3 | 21 | 115 | 93 | 1.24 |
| | 48 | 0.113 | 3 | 20 | 32 | 21 | 1.53 |
| | 48 | 0.225 | 3 | — | 9 | 6 | 1.50 |
| | 48 | 0.450 | 3 | — | 39 | 28 | 1.40 |
| | 48 | 1.350 | 3 | — | 82 | 58 | 1.41 |

[1] These figures represent liters of urine.

The graphs in figure 5 (see also tables 1 and 2) present a comparison of physiological activity to chemical structure, with testosterone as the standard. The comparison is made at the arbitrary point of 1.44 mg. total dose because there are direct determinations either at this point or at the nearby point, 1.50 mg., for all the substances to be considered. The values for relative potency, given above each curve, would of course be different if another point along the abscissa was chosen as a point of reference. They would also be different if a comparison was made with respect to the response of the accessory sex

glands (values along the ordinate), as is indicated by the values for the testosterone propionate and acetate and diacetate. The potencies with respect to total dose are 4.4 for the propionate and acetate, and 3.3 for the diacetate, whereas with respect to a response at 70 mg. weight of prostate the value for the propionate and acetate, 9.6, more than doubles and the diacetate increases to 4.4 (see arrow on ordinate of fig. 5).

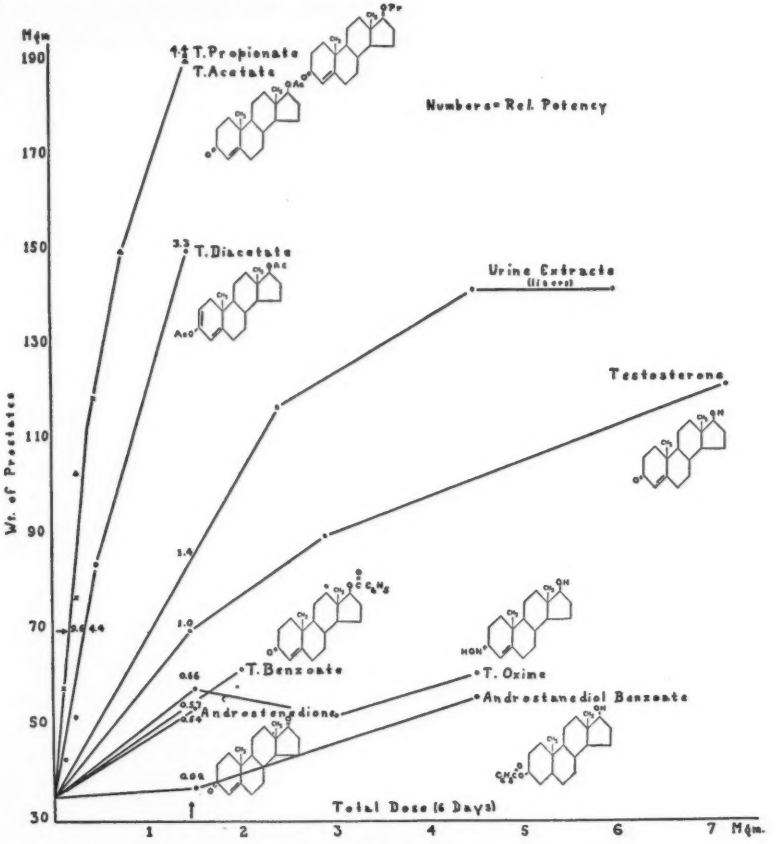


Fig. 5. Comparative efficacy of various androgens. The abscissa values for the urine extract are in liters. The figures on the curves, at abscissa 1.45 and ordinate 70, represent the relative potencies with testosterone as unity.

Androstanediol benzoate is the least active of all the compounds studied. It is only 0.09 as active as testosterone. It differs chemically from testosterone in that it has a completely saturated ring structure and a benzoylated hydroxyl group at carbon atom 3, instead of a free ketone group as in testosterone. Androstenediene which differs from testosterone in only one respect, having a keto group instead of a trans-hydroxyl group at carbon atom 17 is only 0.54 times as active. Benzoylation decreases the potency of testosterone. Testosterone benzoate has only 0.57 the activity of the free compound. This effect is much

more striking in the capon comb test (20, 21). Similar results in the capon test have been found in our laboratory. Injection of 1.0 mg. for 2 successive days produced no response in comb growth in 3 white leghorn capons. Smearing on the capon's comb of 0.1 mg. for 2 successive days produced a doubtful increase of 2 mm. in length plus height of the comb, and this method of assay is known to be 100 times as sensitive as the injection technic (22). These results further illustrate the variation in response due to species differences. It becomes even more important, therefore, to use mammalian test animals whenever possible.

The oxime, which illustrates the effect of the alteration of the 3 keto group, shows not only a decreased activity but also a variable reaction. The testosterone diacetate provides support for the conclusions drawn from the oxime. The alteration of the keto group, even to an easily reversible form such as the enol

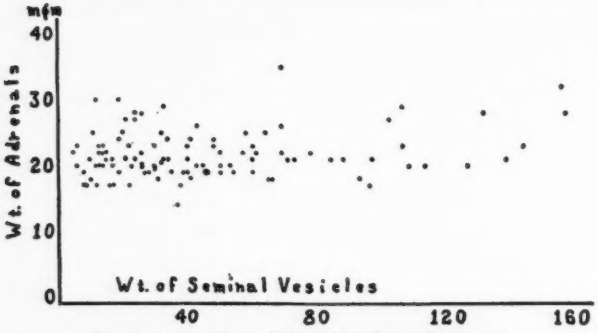


Fig. 6. A comparison of the weight of the adrenals and seminal vesicles of rats treated with androgens.

(23), decreases the activity. Acetylation and propionation of the testosterone molecule greatly enhances its activity, and in approximately the same degree.

*Interval between injection and the efficacy of testosterone propionate, acetate and diacetate.* Since testosterone propionate, acetate and diacetate showed augmented activity it seemed desirable to see if extending the interval between injections would still give the same effect. Various groups of rats were therefore given these substances at 48-hour intervals, and the results compared in terms of total dose with those receiving injection at 24-hour intervals. The results (fig. 4, table 2) demonstrate that as in the case of testosterone there is loss in activity. However, the loss as indicated by the slopes of the curves is not so great as that for testosterone. Of the 3 esters, the propionate is slightly but consistently more active than the acetate. The diacetate also shows a decreased potency.

*Ratio of regeneration of prostate to seminal vesicles.* The degree of regeneration of the prostate to that of the seminal vesicles by these many compounds is worthy of note. Testosterone, testosterone acetate, diacetate and propionate show very nearly the same values for each determination. As compared with the above compounds, the urine extract, however, has produced a much greater

degree of regeneration of the prostate than the seminal vesicles. Androstenedione and androstanediol benzoate indicate a prostate-to-seminal vesicle relationship approaching that of the urine extract, with the exception of the 1.5 mg. dose of the benzoate, which may be neglected because the response is within experimental limits and therefore doubtful.

*Adrenals.* Korenchevsky (24, 25) has found that the adrenals of his castrated rats hypertrophy, and that the hypertrophied glands may be brought back to normal by injection of androgenic material. It is also known that the adrenal cortex produces a compound with androgenic properties (26). Other evidence, which need not be detailed here, suggests cortico-adrenal-gonadal relationship. It therefore seemed advisable, especially in view of work now being completed in this laboratory on the effect of androgens on the survival time of adrenalectomized rats (24) that the adrenals of the rats used in this investigation be studied gravimetrically. The results obtained (fig. 6) show that there is not only no evidence for hypertrophy due to castration but also that androgens causing an increase in weight of the seminal vesicle from 6 to 160 mg. still have no significant effect on the adrenals within the range of the scatter found. Of course this does not eliminate the possibility of histological effects, but it is in direct opposition to Korenchevsky's findings. The weight of the adrenals of our castrate rats ranges from 18 to 31 mg.; the greatest value is still less than one-half that of Korenchevsky's castrate rats. This discrepancy in results might be due to a difference in time of castration. Our rats were castrated just before maturity (38 to 46 days of age) while Korenchevsky's at castration were still quite immature (21 to 26 days). The time of castration with respect to maturity might therefore throw some light on possible adrenal gonadal relationships, and constitutes a problem in itself.

## DISCUSSION

The increase in efficacy of testosterone as a result of shortening the interval between injections is compatible with normal physiological processes. The normal testes must continually secrete into the blood stream a supply of hormone to maintain the accessory sex organs. Consequently, to simulate this state by artificial means, the hormone must be supplied constantly, or else some modification made in procedure or chemical structure, to bring about the same effect.

Miescher et al. (6) have already extended the work of the Laqueur group, and find that the addition of fatty acids enhances the activity of testosterone. The saturated fatty acids seem to be more effective than the unsaturated, and an hydroxyl group enhanced, while several double bonds decreased, the activity. Monhydric alcohols were also effective. The amount and nature of the oil in which the androgen is dissolved (7, 8, 9, 10) likewise affect the activity. While Miescher and co-workers (6, 7, 9) feel that the activation occurs only with androgens containing an hydroxyl carbon atom 17, Parkes and co-workers (8, 10) report activation of androgens containing a keto group instead of an hydroxyl in the 17 position, e.g., androsterone and androstenedione.

The activating effect of the various fatty acids and of the esters is attributed by both of the above laboratories (6, 7, 8, 9, 10) to decreasing the absorption rate of the androgen and thus preventing its rapid destruction.

The greatly enhanced results, obtained with testosterone propionate and acetate, indicate that the decreased effect obtained by esterification in the case of the benzoate is not due to esterification *per se* but to the nature of the esterifying acid. This fact has been beautifully demonstrated, in the meantime, by Miescher and co-workers (7). The marked difference in the effects of the acetate and propionate, on the one hand, and the benzoate, on the other, might be explained by their different rates of hydrolysis. The acetate is more easily hydrolyzed than the benzoate. This difference was made use of by Ruzicka and co-workers (27) in the preparation of the intermediate product, androstenediol —17 benzoate from androstanediol—3 acetate, 17 benzoate for the synthesis of testosterone. Furthermore, the greater ease of hydrolysis of ester groups on the 3 position to those in the 17 position (27, 28, 29, 30) may be used to explain the fact that the benzoates of androsterone (31) estrone (32) and α-estradiol (32) have a delayed and prolonged effect in contrast to testosterone benzoate (20) which has no appreciable immediate or delayed effect.

The difference in the response of the rat and the capon to testosterone benzoate is apparently due to species difference. The rat appears able partly to hydrolyze the testosterone benzoate, while the capon is able to hydrolyze it only very slightly before it is destroyed or inactivated by the body. On the other hand, the rate of hydrolysis of the acetate and propionate is such that it releases the testosterone in a fashion simulating the injections at short intervals. Furthermore, esterification in all probability protects the molecule from destruction as well as decreases the rate of absorption. That the animal body destroys injected androgens has been clearly demonstrated (33) in this laboratory, and the experiment is now being extended to determine whether there is a difference in the amount of destruction of the various compounds of testosterone in comparison to the free form.

The decreased efficacy of the acetate and propionate as a result of increasing the interval of injections is not in complete agreement with Parkes' results (10). He found that the propionate has a greater prolongation effect than either the free form or the acetate, and that one single injection of 2 mg. will produce a maximum effect 10 days later and a very marked one as much as 20 days after the injection. Furthermore, in using a M.E.D. of either the acetate or propionate, the same effect could be produced by dividing the total dose, i.e., twice daily up to once every 5 days. These latter findings are not in complete accord with the results reported here. The increase of the injection interval, from 1 day to only 2 days, shows an appreciable decrease in efficacy. Also, the slopes of the curves indicate that, even though the dose should be appreciably increased, there would still be a decrease in efficacy as a result of lengthening the interval between injections. It must be noted, however, that there is considerable difference in the amount of oil used in comparison to that used by Parkes. A total of 5 cc. of oil was administered with the hormone by the latter, whereas in the experiments reported here a total of only 1.8 cc. was

used in the daily injection, and even less, 0.9 cc., in the 48 hourly injection experiments.

This might be considered a reason for the decrease in efficacy due to lengthening the interval between injections, but it becomes a moot point in view of Parkes' report (10) that the amount of oil has no appreciable effect in the efficacy of these esters. In the light of the above findings, it seems that Parkes' conclusions, with respect to quantity of oil or else the relationship of degree of efficacy to interval between injections, are in need of some modification.

The relatively high potency of the urine extracts, not to be attributed to the activity of its known androgenic compounds, cannot be explained at present, but might be due to several possible factors. *a)*. There is an androgenic compound other than androsterone or dehydroandrosterone which is more potent than either of these compounds. *b)*. A proper combination of androsterone and dehydroandrosterone possibly enhances the effects of each other. *c)*. An accessory substance is present which enhances the activity of either androsterone or dehydroandrosterone or both. If so, it must be different from the activating substances, known to be fatty acids, of Miescher, his co-workers and Parkes. The urine extract by virtue of its method of preparation is neutral. Likewise *d)*. the enhancing effect cannot be due to estrone or a related compound because of the neutral character of the extract. However, the extracts as prepared contain estrogenic activity (34) which is probably due to the dehydroandrosterone (35, 36). *e)*. We have been able to enhance the activity of testosterone 50 per cent by the addition of a neutral fraction obtained from male castrate dog urine which showed no androgenic activity by itself. *f)*. The urine extract has a much greater effect than testosterone on the regeneration of the prostate; and this effect is simulated to a certain extent by androstanediol benzoate, androstendione and possibly testosterone benzoate.

## SUMMARY

The efficacy of various androgens was determined by the gravimetric rat assay method. The following order of increase in potency was found: androstanediol benzoate," androstenedione, testosterone oxime, testosterone benzoate, testosterone, urine extract, testosterone diacetate, testosterone acetate, and testosterone propionate. Lengthening of the interval between injections resulted in a decrease in efficacy. The marked difference in the activity of the acetate, diacetate and propionate to the benzoate is attributed to the ability of the animal organism to hydrolyze the former compounds more satisfactorily. The relatively high potency of the urine extract is believed to be due to some factor or factors other than the known androgenic constituents.

The weights of the adrenals of the rats used in this study were not affected.

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# THE INTERRELATION OF PITUITARY AND ADRENAL IN THE CONTROL OF CARBOHYDRATE LEVELS IN THE RAT[1]

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The fact that hypophysectomized animals are unable to maintain their fasting carbohydrate levels has been well established (1 to 5). A preliminary report from this laboratory (6) has shown that the injection of suitable APE during the fasting period will maintain muscle glycogen of hypophysectomized rats at normal or supra-normal levels, and that the adrenals are not necessary for this action of the extract. This investigation is a comparison of the ability of crude anterior lobe extracts, adrenocorticotropic hormone and adrenal cortical extracts to maintain fasting carbohydrate levels of hypophysectomized, hypophysectomized-adrenalectomized, and adrenalectomized animals.

## EXPERIMENTAL

All chemical analyses and operative procedures were carried out as previously reported (6). Two different adrenocorticotropic preparations were used in these experiments, BA-2 and M 222-1. BA-2 was prepared from whole sheep pituitaries by the method of Lyons (7), and was free of growth promoting effects when tested at 10 mg. daily loses in hypophysectomized rats. It was also free of thyreotropic and gonadotropic effects in the immature squab. It contained 1000 subcutaneous units (8) of lactogenic per gm., and 40 U (9) (the level producing 50 per cent hypertrophy in the adrenals of 24-day-old male rats after 3 daily injections) of adrenocorticotropic hormone per gm. Extract M 222-1 was prepared by the same method from beef anterior lobes, and assayed essentially the same.

Two different standard alkaline preparations were also used,—W 6027 and B 479, prepared as previously reported (6). Their assay was essentially the same: 180-185 U of growth hormone, 100 subcutaneous U of lactogenic, and 10 U of adrenocorticotropic per gm. In addition, they were rich in thyreotropic and contained some gonadotropic hormones. It should be noted that these extracts contained approximately one-fourth of the adrenocorticotropic potency and one-tenth of the lactogenic potency of BA-2 or M 222-1.

The study of the effectiveness of B 479 and BA-2 in maintaining the muscle glycogen of the 24-hour fasted hypophysectomized rat is summarized in table 1. All injections were made intraperitoneally. In these experiments the extracts were given only during the fasting period, and injections were

[1] Aided by the Presnell Fund for the study of the metabolic relations of the anterior hypophysis, contributed by Robert R. Presnell, Frank Tuttle, Arthur Stebbins and Mrs. Gordon Kahn of Los Angeles.

TABLE 1. CARBOHYDRATE LEVELS OF 24-HOUR FASTED HYPOPHYSECTOMIZED RATS TREATED WITH DIFFERENT AMOUNTS OF STANDARD ALKALINE EXTRACT (B 479) AND ADRENOCORTICOTROPIC HORMONE (M 222-1 and BA-2). INJECTED ONLY DURING THE FASTING PERIOD

| Total amount of extract used per 100 gm. body weight | Muscle Glycogen mg. % | | Liver Glycogen mg. % | | Blood Glucose mg. % | |
|---|---|---|---|---|---|---|
| | Mean | Range | Mean | Range | Mean | Range |
| 1.0 mg. of B 479 (5)[1] | 506 | 445–566 | 16 | 11–21 | 52 | 44–59 |
| 0.75 mg. of B 479 (4) | 437 | 408–466 | 12 | 8–14 | 53 | 37–61 |
| 0.50 mg. of B 479 (5) | 432 | 404–461 | 15 | 7–19 | 50 | 39–65 |
| 15.0 mg. of BA-2 (5) | 485 | 370–559 | 15 | 9–22 | 65 | 58–77 |
| 10.0 mg. of BA-2 (5) | 457 | 359–527 | 16 | 12–23 | 64 | 55–77 |
| 5.0 mg. of BA-2 (5) | 367 | 319–422 | 20 | 16–23 | 69 | 45–97 |
| 5.0 mg. of M 222-1 (2) | 330 | 307–354 | 9 | 9– 9 | 61 | 60–63 |
| 13.4 mg. of M 222-1 (3) | 358 | 295–437 | — | — | 44 | 34–57 |

[1] Number of observations.

made at 24, 20, and 4 hours previous to sacrifice. Inspection of the data reveals the fact that while 1.0 mg. of B 479 per 100 gm. body weight produces complete maintenance of muscle glycogen, it requires 15 times as much BA-2 to produce a similar maintenance. These comparisons are valid because previous work (10) has shown that in a narrow range near the M.E.D. level there is a quantitative relation between the amount of extract administered and its 'glycostatic' effect. This means that there is a 60-fold discrepancy between the 'glycostatic' and adrenocorticotropic potency of these 2 extracts. This difference is not due to a species difference in the glands used as starting material, because M 222-1 is even less active in the 'glycostatic' factor than is BA-2. M 222-1, like B 479, was prepared from beef anterior lobes.

Further evidence that the action of the crude extract is not through the adrenal is furnished by the following experiments in hypophysectomized-adrenalectomized rats. These double operations were carried out as follows:

TABLE 2. CARBOHYDRATE LEVELS OF 24-HOUR FASTED HYPOPHYSECTOMIZED AND ADRENALECTOMIZED RATS TREATED WITH STANDARD ALKALINE EXTRACT

| Operation and Treatment | Muscle Glycogen mg. % | | Liver Glycogen mg. % | | Blood Glucose mg. % | |
|---|---|---|---|---|---|---|
| | Mean | Range | Mean | Range | Mean | Range |
| Untreated Hypophysectomized (11)[1] | 317 | 251–400 | 15 | 10–22 | 50 | 41–63 |
| Hypophysectomized (4) 10 mg. of W 6027 in the 72 hr. preceding sacrifice | 515 | 452–619 | 21 | 20–22 | 42 | 38–44 |
| H-A[2] (6) 10 mg. of W 6027 in the 48 or 72 hr. preceding sacrifice (6) | 545 | 427–640 | 17 | 5–26 | 35 | 28–45 |
| Hypophysectomized (5) 1.0 mg. of B 479 per 100 gm. body weight in 24 hr. preceding sacrifice | 506 | 445–566 | 16 | 11–21 | 52 | 44–59 |
| H-A 1.5 mg. B 479 per 100 gm. body weight in 24 hr. preceding sacrifice (3) | 480 | 433–528 | — | — | 43 | 34–49 |
| Untreated normal (13) | 500 | 480–545 | 23 | 13–33 | 79 | 60–89 |

[1] Number of observations.
[2] H-A—Hypophysectomized-adrenalectomized.

one week previous to hypophysectomy, the right adrenal was removed (in a few cases hypophysectomy was done at the same time); 11 to 19 days after hypophysectomy the left adrenal was removed, and therapy with standard alkaline extract was started. The data are shown in table 2, which also indicates the length of treatment. Most striking is the complete maintenance of muscle glycogen in the absence of both adrenals. No cortical therapy was given, because all animals were sacrificed before insufficiency symptoms developed after removal of the second adrenal. It would hardly seem that the 'glycostatic' activity of this extract is mediated through stimulation of the adrenal cortex.

It cannot be emphasized too strongly that the above experiments, as well as those immediately preceding, are acute experiments; i.e. in no case were

TABLE 3. EFFECT OF ADRENAL CORTICAL EXTRACT ON CARBOHYDRATE LEVELS OF ADRENALECTOMIZED AND OF HYPOPHYSECTOMIZED RATS[1]

| | Muscle Glycogen mg. % | | Liver Glycogen mg. % | | Blood Glucose mg. % | |
|---|---|---|---|---|---|---|
| | Mean | Range | Mean | Range | Mean | Range |
| Untreated, adrenalectomized 3–4 days after operation (6)[2] | 477 | 441–538 | 14 | 12– 16 | 56 | 43– 63 |
| Hypophysectomized treated with eschatin and salt (6) | 335 | 299–390 | 9 | 5– 14 | 63 | 49– 72 |
| Untreated, unfasted, adrenalectomized with symptoms of adrenal insufficiency, 10–20 days after operation (4) | 325 | 316–340 | 20 | 9– 37 | 57 | 24– 90 |
| Unfasted adrenalectomized treated with eschatin and salt (4) | 536 | 490–573 | 3080 | 2900–3210 | 116 | 104–122 |
| Adrenalectomized treated with eschatin and salt (4) | 443 | 417–458 | 4 | 3– 7 | 61 | 57– 64 |

[1] All fasted 24 hours except as noted.
[2] Number of animals in the group.

the extracts given for a longer period than 72 hours, and in most cases they were given only during the fasting period. A most striking contrast appears between the effect of extracts given acutely in this manner and those given over longer periods of time (11).

Previously it has been reported from this laboratory (2) that within 24 hours after operation fasting hypophysectomized rats are unable to maintain their muscle glycogen level. Table 3 shows that this defect does not appear so rapidly after adrenalectomy, and to confirm Corey (12), it is shown that adrenal cortical therapy[2] adequate to maintain normal muscle glycogen levels in adrenalectomized rats is without effect on hypophysectomized rats.

## SUMMARY

Several of the previous findings indicate that the pituitary influences the maintenance of fasting carbohydrate levels in the rat directly, rather than through the mediation of the adrenal.

[2] Eschatin was supplied through the courtesy of the Parke, Davis Company.

Hypophysectomized rats immediately after operation become unable to maintain their fasting muscle glycogen levels, while adrenalectomized rats do not develop this defect for several days after operation. The fasting muscle glycogen of adrenalectomized rats (proven to develop insufficiency symptoms) is maintained within normal limits by cortin and salt therapy; this treatment is without effect on the hypophysectomized rat. Crude anterior lobe extracts in the acute test maintain the fasting muscle glycogen level of hypophysectomized rats in the absence of both adrenals.

Purified adrenocorticotropic hormone has only one-fifteenth the potency of crude extracts for maintaining fasting muscle glycogen in the acute test. As pointed out before, there is a 60-fold discrepancy between the 'glycostatic' and adrenocorticotropic activity of the two extracts. This factor acting independently of the adrenals has not been identified with any of the known anterior lobe hormones.

The author wishes to acknowledge his debt of gratitude to Dr. Herbert M. Evans, whose inspiration, help and guidance suggested and made possible this work.

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# A MALE SEX-STIMULATING AND FEMALE SEX-REPRESSING FRACTION FROM THE ADRENAL GLAND[1]

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Recent work on the chemistry of the adrenal cortex has resulted in the isolation of several crystalline substances, some of which have been reported physiologically active in maintaining the life of bilaterally adrenalectomized animals. Reichstein (1), Kendall (2), Wintersteiner (3), and their collaborators have shown that some of these crystalline compounds have the ring structure characteristic of the sterols and sex hormones. Kendall (4) has reported that one of his crystalline substances stimulated comb growth in the capon.

Many workers have indicated a relationship between the adrenals and sex function. Adrenal cortical hyperfunction, particularly that resulting from tumor growth, stimulates the secondary sex characteristics not of the same sex but of the opposite sex. Women exhibit male hair distribution, deep voice, amenorrhea, atrophic breasts, and atrophic genitalia, while men exhibit a female type of obesity, breast hypertrophy, atrophy of testes and absence of libido. Saphir (5), Cahill (6), Lisser (7), Goldzieher (8) and their collaborators have reported some of this work. Still others (9, 10, 11) have reported intsances of precocious sexual maturity produced by cortico-adrenal extract.

In adrenal insufficiency there seems to be a reduction of the sexual functions with resultant gonadal atrophy (12, 13, 14). All of these observations indicate a close relationship between the adrenals and sexual function.

With respect to the preparation of adrenal extracts containing fractions which are responsible for the above phenomena, little work has been reported. Ovarian stimulation in rats (luteinization and endometrial hyperplasia) by an adrenal extract has been reported by Allen and Bourne (15), and Casida and Hellbaum (16). Hodler (17) has recently made a report concerning an alkaline aqueous extract of the adrenal cortex, which has a masculinizing effect on the genital organs of normal and ovariectomized female and on castrated male guinea pigs.

We report here the results of another fraction of the adrenal cortex used experimentally by the Research Department of the Pottenger Sanatorium. In a group of children receiving adrenal cortex extract, it was observed that a large number increased in weight during treatment. This led us to search for a growth-promoting factor, which ended in discovering a female sex-

[1] Read at the twenty-first Annual Meeting of the Association for the Study of Internal Secretions, Atlantic City, N.J., June 7, 1937.

repressive and a male stimulative factor. Klein (18) prepared a saline extract of adrenal cortex which produced a stimulation in the development of the male sex organs and an inhibition of the female sex organs of rats. Our extract was prepared by an entirely different method, as described in the experimental procedure.

## EXPERIMENTAL

Adrenal cortex extract was prepared by the method of Swingle and Pfiffner (19). The benzene insoluble fraction (originally discarded) was treated by a modification of the Wallen-Lawrence and van Dyke (20) procedure for the extraction of gonad-stimulating substances from anterior pituitary powder and from pregnancy urine. Briefly, the procedure used is as follows. To the benzene insoluble fraction was added 500 cc. of N/5 acetic acid and 500 cc. of N/5 sodium acetate. The mixture was thoroughly agitated for 15 minutes by means of a mechanical mixer and then allowed to stand for 48 hours in the ice chest (O°C.). The material was filtered and the filtrate concentrated to a volume of 300 cc. by vacuum distillation. The concentrated solution was poured into 10 volumes of 95 per cent ethyl alcohol (containing 5 cc. of acetic acid per liter) and placed in an ice chest (—25 to —30°C.). A dark, gummy precipitate settled out. The solution was removed by filtration, the precipitate dissolved in water, filtered, and poured into 10 volumes of 95 per cent alcohol. The procedure was repeated from 4 to 7 times in order to remove inert material. In the last 2 reprecipitations, the acetic acid was not added to the alcohol. A light brown colored powder was isolated which was water soluble, alcohol insoluble, and apparently heat stable at 60°C. The color may be completely removed by treatment with charcoal, although care must be used, for the latter compound adsorbs some of the active portion. The powder is made up in physiological saline, filtered through permutit in order to remove the last traces of epinephrine and sterilized by Berkefeld filtration. The concentration is such that 1 cc. of the above solution is equivalent to 66.6 gm. of whole gland.

Evans and Simpson (21) have shown that pituitary growth hormone administered to rats caused not only a marked increase in weight but also stimulated sex activity. Since increase in the weight of rats is easily observed, the above-mentioned fraction was assayed for its growth-promoting property by this method. Female rats were given 0.5 cc. injections (subcutaneous) of this material daily for 20 days. The control rats were litter mates of the test animals and were untreated. Both control and test animals were weighed at 5-day intervals. At the end of the 20-day period, the animals were sacrificed and the uterus, fallopian tubes and ovaries were removed. Photographs were taken, and in later experiments the organs were weighed. Male rats were subjected to the same procedure and a study was made of the testicles of these animals.

As the results obtained from this male-stimulative, female-repressive fraction (hereafter called the *S-S* fraction) were rather unusual, the cortical extract was assayed by the same procedure. In our modification of the Swingle and

Pfiffner (19) procedure, the following fractions are obtained from the benzene residue: *A)*, the alcohol-soluble fraction; *B)*, the alcohol-insoluble fraction obtained by cooling the alcohol soluble to —25 to —30°C.; *C)*, the water-soluble fraction. The effect of each of these fractions and one commercial product (eschatin)[2] was studied on both male and female rats.

A total of 54 female rats was used in this experiment: 36 of them were treated with the *S-S* fraction, 6 with the alcohol-soluble fraction *A)*, 4 with the alcohol-insoluble fraction *B)*, 4 with the water-soluble fraction *C)*, and 4 with eschatin.

TABLE 1. FEMALE RATS.

| | Rat No. | Body Wt. gm. | Wt. of Uterus gm. | Wt. of Uterus / Body weight % | Average % |
|---|---|---|---|---|---|
| *S-S* Fraction, 159–60. | | | | | |
| Test | S54 | 109 | 0.144 | 0.132 | |
| | S80 | 114 | 0.199 | 0.175 | 1.53 |
| Control | S50 | 102 | 0.190 | 0.186 | |
| | S7 | 118 | 0.170 | 0.144 | 1.65 |
| *S-S* Fraction, 159–60. | | | | | |
| Test | M1 | 88 | 0.136 | 0.155 | |
| | L2 | 93 | 0.225 | 0.242 | 0.196 |
| Control | M20 | 90 | 0.224 | 0.249 | |
| | L13 | 110 | 0.198 | 0.180 | 0.214 |
| Water-soluble Fraction *C)*. | | | | | |
| Test | O4 | 62 | 0.049 | 0.079 | |
| | Q50 | 52.5 | 0.028 | 0.053 | 0.066 |
| Control | O5 | 58.1 | 0.035 | 0.061 | |
| | Q31 | 59.8 | 0.043 | 0.072 | 0.066 |
| Eschatin | | | | | |
| Test | O3 | 31.3 | 0.026 | 0.082 | |
| | T60 | 52.7 | 0.028 | 0.054 | 0.068 |
| Control | O10 | 47.0 | 0.032 | 0.067 | |
| | T65 | 52.9 | 0.034 | 0.063 | 0.065 |
| Alcohol-soluble Fraction *A)*. | | | | | |
| Test | 1A-20 | 103 | 0.165 | 0.161 | |
| | 1B-50 | 117 | 0.182 | 0.155 | 0.158 |
| Control | 1A-30 | 104 | 0.202 | 0.195 | |
| | 1B-13 | 113 | 0.220 | 0.195 | 0.195 |

Thirty-four male rats were used and divided as follows. 16 were treated with the *S-S* fraction, 6 with the alcohol soluble fraction *A)*, 4 with the alcohol-insoluble fraction *B)*, 4 with the water-soluble fraction *C)*, and 4 with eschatin. Half of the number of rats in each group were test animals, the other half control animals.

## RESULTS AND DISCUSSION

### *Female Rats (Gross Observations)*

The uteri of the test animals on the *S-S* fraction were long and slender, and showed marked atrophy as compared to those of the control animals. In a large number of test animals the horns were 5 or 6 mm. longer than those

[2] Part of the eschatin was donated by Parke Davis Company.

of the controls, while the diameter of the uterus (at the base) was from one-third to one-half of that of the control animals. In a few of the test animals, hyperemia of the ovaries was noted.

With the alcohol-insoluble fraction *B)* the reverse phenomena were noted,

TABLE 2. MALE RATS.

| | Rat No. | Body Wt. gm. | Wt. of Testes gm. | Wt. of Testes / Body Wt. % | Average % |
|---|---|---|---|---|---|
| *S-S* Fraction, 120. | | | | | |
| Test | F9 | 87 | 1.30 | 1.49 | |
| | F6 | 95 | 1.60 | 1.68 | |
| | H50 | 81 | 1.10 | 1.36 | |
| | G40 | 95 | 1.70 | 1.77 | 1.57 |
| Control | F5 | 110 | 1.70 | 1.54 | |
| | F4 | 94 | 0.70 | 0.74 | |
| | H55 | 96 | 1.20 | 1.25 | |
| | G44 | 89 | 1.60 | 1.79 | 1.33 |
| *S-S* Fraction 159–60. | | | | | |
| Test | R84 | 118 | 1.63 | 1.38 | |
| | R70 | 129 | 1.74 | 1.35 | 1.37 |
| Control | R59 | 107 | 1.55 | 1.45 | |
| | R90 | 131 | 1.42 | 1.08 | 1.27 |
| *S-S* Fraction 159–60. | | | | | |
| Test | M11 | 104 | 1.58 | 1.52 | |
| | M22 | 101 | 1.56 | 1.54 | 1.53 |
| Control | M10 | 101 | 1.49 | 1.48 | |
| | M21 | 95 | 1.38 | 1.45 | 1.47 |
| Alcohol-insoluble *B*). | | | | | |
| Test | L5 | 124 | 1.52 | 1.23 | |
| | L9 | 111 | 1.55 | 1.40 | 1.32 |
| Control | L7 | 128 | 1.61 | 1.26 | |
| | L60 | 120 | 1.16 | 0.97 | 1.12 |
| Water-soluble *C*). | | | | | |
| Test | P22 | 59.8 | 0.62 | 1.03 | |
| | P23 | 50.0 | 0.51 | 1.01 | 1.02 |
| Control | P30 | 57.2 | 0.58 | 1.02 | |
| | P13 | 55.5 | 0.36 | 0.65 | 0.84 |
| Eschatin | | | | | |
| Test | N1 | 87.0 | 1.04 | 1.19 | |
| | T55 | 48.8 | 0.42 | 0.85 | 1.02 |
| Control | N2 | 96.6 | 1.37 | 1.41 | |
| | T15 | 57.5 | 0.54 | 0.94 | 1.18 |
| Alcohol-soluble *A*). | | | | | |
| Test | 1A-7 | 158 | 2.52 | 1.60 | |
| | 1B-11 | 167 | 2.73 | 1.63 | |
| | 1C-26 | 144 | 2.18 | 1.52 | 1.58 |
| Control | 1A-4 | 140 | 2.30 | 1.65 | |
| | 1B-8 | 142 | 2.28 | 1.61 | |
| | 1C-29 | 86.2 | 1.08 | 1.26 | 1.51 |

i.e., the uteri of the test animals were short and thick as compared to those of the controls. No significant change was noted in the uteri of the test animals as compared to those of the controls when the test animals were treated with eschatin or with the water-soluble fraction *C)* which is comparable to eschatin.

*Weight observations.* Table 1 shows the weights of the rats and of the sex-organs of a small group of female rats. The lack of a suitable balance pre-

vented the recording of these weights in the early experiments. The weight of the uterus was calculated in terms of per cent of body weight. As the chart shows, in the average percentage of each group, the uterus of the test animal on the *S-S* fraction was smaller than that of the control. It is a small difference yet a significant one. In the rats treated with the water-soluble fraction *C)* and with eschatin, these percentages were practically equal.

The weights of both test and control animals increased during the 20-day treatment period. Variations in individual rats were noted but, based on group averages, these changes were insignificant.

*Histological Observations.* Microscopic examination of sections of uteri revealed the following facts. The control animals showed more follicular development than the test animals. In several rats a large amount of luteinization was found, but the control animal showed a much more highly developed type of uterus. The chief observation in the uteri of the test animals was a degeneration of underlying epithelial cells.

### *Male Rats (Gross Observations)*

No marked difference was observed in the testicles of the test and control animals other than that of size, which will be discussed under weight changes.

*Weight Observations.* Table 2 gives the weight chart of all male rats used in this experiment. In 6 of 7 groups of male rats, there was an increase in the weight of the testicles (average weight of each group) of the test animals as compared to that of the controls. In the test animals, the average weight of the testicles per group ranged from 0.57 to 2.73 gm. and in the controls from 0.47 to 2.30 gm. Although this weight change is small, the significant fact is that a positive correlation was found consistently in the test animals. The variation in the weights of the test and control animals (average weight per group) was insignificant. In the sixth group, eschatin was used, and a negative correlation was found, i.e., the testicles of the control rats were heavier than those of the test animals.

*Histological Observations.* Microscopic examination of sections of the testicles revealed the following facts. In the test animals, the tubules showed a greater activity than in those of the controls, and the test animals showed a more mature spermatogenesis.

## DISCUSSION

The histological changes observed, both in the male and female rats, seem to be functional rather than structural. Continued treatment might bring about a pathological condition. The age of the rats seems to play an important rôle. In the female rats, the atrophy is much more marked in the mature than in the young animal (5 to 8 weeks of age). In the young animal, the *S-S* fraction may cause only a temporary inhibition of uterine and ovarian growth. In the male rat the stimulating action is about equal in both young and old animals.

One interesting observation was the homosexual tendencies displayed by these rats. It was more pronounced in the male than in the female animals.

The test animals showed erection of the penis as soon as they were handled, while the controls did not. Broster and Vines (22) have isolated a crystalline compound from the urine of a patient suffering from hypersecretion of the adrenal. This compound seems to be specific to virilism, for the patient who exhibited homosexual tendencies before operation returned to normalcy afterwards.

The female sex-repressive fraction is confined in the discard portion of the adrenal extract prepared by the method of Swingle and Pfiffner (19). With the exception of the alcohol-insoluble fraction which seems to possess stimulative properties, the fractions possessing cortical activity are inactive in producing this repressive effect.

The male-stimulative effect is obtained from the various fractions of the adrenal gland, both the cortical and noncortical. It is probably related to the chemical compound cortin.

## SUMMARY

By purifying the benzene discard of the Swingle and Pfiffner method of extraction of adrenal glands, by a technique described, a white amorphous powder is obtained. When tested on female rats, it causes a functional atrophy of the uteri and the uterine horns. When tested on male rats, it causes an increase in weight of the testes and an increase in spermatogenesis. These bisexual effects are not obtained from the benzene soluble fractions. In one fraction the opposite effect was obtained on the female tract.

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# AN ORALLY-ACTIVE SEX-MATURATION FRACTION FROM THE ADRENAL GLAND

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Cryptorchism, following the report of Bevan (1) was formerly looked upon in America as a condition which only the surgeon could correct. Shapiro (2) in 1930 showed that the anterior pituitary-like hormone of pregnancy urine, in certain cases of undescended testicles, brought about rapid descent. Engelbach (3) noted that patients with thyroid deficiency and cryptorchism often obtained a descent of the testicles from the administration of thyroid extract. Drake (4) reported a series of boys whose testes responded to the stimulation of puberty. The gonadotropic hormone of the anterior pituitary has been used successfully by Werner (5) and his associates.

This report is based on the observation of boys who, with one exception, have come under my care for a condition that is rarely looked upon as a developmental disease, namely allergy, particularly asthma. In studying these children we were early impressed by the frequency of undescended testicles and also the apparent smallness of the testes in others who presented no signs of cryptorchism. These facts coupled with similar observations of the sexual development in girls, as well as other clinical and experimental data, led the authors to suspect that childhood allergy, at least in the severe form, may be associated with a failure of factors which are responsible for maturation. In our series of patients we also found, in about 30 per cent of cases studied, a hypothyroid condition as evidenced by delayed bone growth. We obtained a history of thyroid, or so-called 'ovarian trouble' in well over 50 per cent of the mothers of these children.

These observations have extended over a period of 3 years, and each of the children discussed was subjected to a thorough physical examination. Precautions were taken to make sure that neither cold nor psychic factors were responsible for their apparent cryptorchism. This report includes no cases in which testicles could be brought into the scrotum by manual traction at first examination.

The adrenal gland possesses powerful growth-stimulating factors. These factors are not all cortin, although cortin is one of them. There seem to be several sex fractions in the adrenal that may be chemically different. The fractions herein discussed not only affect migration of the organ, but also its size.

The extract used in this work is produced by taking whole adrenal glands through the 2 alcohol extractions, and the benzene extractions of the original Swingle and Pfiffner method (6, 7). The residue resulting, when the benzene is evaporated, is taken up in alcohol or glycerine. Whole glands are used ex-

clusively. The whole gland equivalent given to children varies with their ages. Children from 1 to 3 years of age usually receive 1.25 gm. equivalent 3 times a day by mouth; 3 to 6 years of age 2.5 gm. equivalent 3 times a day; 6 to 10 years of age 5 gm. equivalent 3 times a day; and over 10 years of age, 10 gm. equivalent 3 times a day.

Inasmuch as most of these patients were under treatment for their allergic condition, the descent of the testes was a secondary matter, and the observations were probably not always made as soon as the testicles actually completed their descent. As noted, the earliest descent occurred in 2 months, the latest in 17 months, although the latter observations were undoubtedly delayed. The average time was about 9 months.

The boys ranged in age from 2 to 13 years. In all, 9 boys were observed. Three presented bilateral intra-abdominal testes, one presented a unilateral intra-abdominal testicle. In all instances of intra-abdominal testes full descent was obtained. Three boys presented both testes in the inguinal canal, and complete descent of these inguinal testes was observed. Two boys had but one testicle in the canal. In one instance, in which the testicle descended into the scrotum, testicular and penis growth was still retarded after 17 months. In the other case, the testicle grew to normal size, although it did not fully enter the scrotum, and, as the patient says, "It usually rides just below my abdomen if I push it out in the morning." At night there is a tendency for it to remain just inside the external ring. Consequently, this patient cannot be listed as a complete success. Something apparently draws this testicle back into the inguinal canal.

Only a brief history of the pertinent facts relating to the condition of the testes will be given. No attempt will be made to classify the endocrinopathies which these boys show. However, as stated above, they were all sexually underdeveloped and highly allergic.

## Cases

*Bilateral, intra-abdominal testes. Case 10670. J. E. B.,* aged 2 years. At the beginning of treatment, Oct. 7, 1935, neither testicle could be made out on examination. On Oct. 15, 1936, both testes had fully descended in one year, and were about normal in size. Extract equivalent to 1.25 gm. of fresh gland was given orally 3 times a day.

*Case 10656. J. A.,* aged 3 years. At the beginning of treatment on Sept. 17, 1935, neither testicle could be palpated, and the penis was small. On July 2, 1936, the right testicle had fully descended; the left was in the canal. On March 1, 1937, both testes had fully descended, and their size was about normal for the age. The descent occurred in 17 months. Extract equivalent to 1.25 gm. of fresh gland was given orally 3 times a day.

*Case 10806. R. D.,* aged 9½ years. At the beginning of treatment on June 29, 1936, neither testicle was palpable. On Sept. 17, 1936, both testes had entered the scrotum. On Nov. 24, 1936, both testes were still in the canal but could be pushed into the scrotum. On March 4, 1937, the testes were still small and in the scrotum. The descent occurred in 8 months. Extract equivalent to 5 gm. of fresh gland was given orally 3 times a day.

*Unilateral intra-abdominal testicle. Case 10850. J. G.,* aged 2 years, 3 months. At the beginning of treatment on Sept. 17, 1936, there was a large accumulation of

fat over the mons. The left testicle was well developed, but not quite in the scrotum. The right testicle could not be made out. On May 17, 1937, the scotum was well developed, with no excess fat. Both testes were in the scrotum, and about the same size, 1.3 x 0.8 cm. The penis measured about 4 cm. Descent occurred in 8 months. Extract equivalent to 2.5 gm. was given orally 3 times a day.

*Bilateral inguinal testes. Case 10635. S. V. D.,* aged 3 years. At the beginning of treatment on July 26, 1935, both testes were still in the canal. On Dec. 18, 1936, both testes had fully descended. They were smaller than normal. Descent occurred in 17 months. Extract equivalent to 3.3 gm. was given orally 3 times a day.

*Case 10797. D. O.,* aged 7 years. At the beginning of treatment on June 9, 1936, both testes were above the external ring and very small. On July 22, 1936, both testes were high in the scrotum. On Nov. 25, 1936, both testes were well in the scrotum, but apparently not increased in size. Descent occurred in 5 months. Extract equivalent to 5 gm. of fresh gland was given orally 3 times a day.

Fig. 1. *A.* Photograph of genitals of patient, *case 10913*, March 5, 1937. Taken at a distance of 42 inches. *B.* Same patient, April 9, 1937. Taken at a distance of 30 inches.

*Case 10856. C. W. H.,* aged 13 years. At the beginning of treatment on Oct. 12, 1936, both testicles (small) were in the inguinal canal. There was little pubic hair. On Dec. 14, 1936, both testes were high in the scrotum. On April 1, 1937, both testes were well in the scrotum, the voice had changed, pubic hair was well developed, and the testes measured about 3 x 2 cm. Descent occurred in 2 months into the scrotum. Extract equivalent to 10 gm. fresh gland was given orally 3 times a day.

*Unilateral inguinal testes. Case 10708. G. S. C.,* aged 11 years. At the beginning of treatment, Dec. 11, 1935, the right testicle was in the scrotum, the left in the canal just below the internal inguinal ring, the testicles were small. On July 7, 1936, both testes were in the scrotum and had greatly increased in relative size, although still small. On Sept. 22, 1936, both testes had fully descended and had possibly increased in size. On Dec. 17, 1936, the testes measured about 1.5 x 1 cm. The penis was still small, being 3 cm. in length. Descent occurred in 17 months. Extract equivalent to 6.7 gm. of fresh gland was given orally 3 times a day.

*Case 10658. C. M.,* aged 12 years. At the beginning of treatment on Sept. 20, 1935, the right testicle was in the scrotum, the left one was above the ring and could not be brought into the canal. The testes were very small. On Jan. 5, 1936, the right testicle was in the scrotum, while the left testicle at the external ring could be pushed into the scrotum; the testes had increased in size; the pubic hair

had begun to develop. On Feb. 28, 1936, the left testicle could be pushed entirely through the ring. On Feb. 2, 1937, the size of the testicles was about normal for a boy of his age; the left testicle could be brought completely into the scrotum, but seemed to have some tissue ahead of it. It had not descended after 17 months. Treatment had been given only one third of this time. Extract equivalent to 6.7 gm. of fresh gland was given orally 3 times a day.

Figure 1 shows a case of a mentally retarded Jewish child who from birth had almost no external genital development. In fact, the Rabbi told the parents he could not circumcise the infant. In the case of this boy, who at the age of 16 had testicles that measured 0.8 x 1 cm., treatment with extract representing 10 gm. equivalent of whole gland 3 times daily for a period of 30 days increased the size of the testicles to 2 x 3 cm. His intellectual improvement was remarkable during the same period.

This extract seems to stimulate growth of testicular size of boys under 4 years of age and those at the age of puberty. Boys between the ages of 4 and 12 years do not seem to experience the same stimulation of testicular growth, although migration of the organs is apparently initiated at any time. To date we have not observed a case of cryptorchism after puberty. Nor have we noted any stimulating sex effects in boys with normal genital development.

There seems to be a very definite relationship between the adrenals, skeletal size and maturation. Maturation seems to be influenced in severe allergy. Changes in the rate and degree of maturation and the recovery from allergy also seem to be related. Even though the adrenal extract is unable to restore a normal maturation balance, the severity of the allergy is usually lessened, if not entirely abated.

SUMMARY

The residue from the benzene fraction obtained by the Swingle and Pfiffner method of extracting adrenal glands contains a factor which is gonadotropic and effective in restoring the physiologic balance of allergic children. It may or may not be cortin.

Of 9 boys with cryptorchism the testes of 3 were both located in the abdomen; 1 boy had but one in the abdomen; 3 had both testes located in the inguinal canal; and 2 had but one testicle in the canal. Complete descent was obtained with 8 boys. One boy presented a single testicle in the inguinal canal which failed to descend completely.

In this group a general enlargement of the testes was noted, although normal size was not attained in all cases. Stimulation of the size of the testes seems to vary with the age of the boy.

A case of definitely arrested development of the external genitalia is presented in which marked stimulation was obtained by 30 days' treatment with the cortical adrenal extract.

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# EFFECT OF CORTIN ON THE EXCRETION OF ELECTROLYTES[1]

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In an earlier paper (1) from this laboratory it was shown that the injection of large amounts of cortical extract into normal human beings caused marked changes in the excretion of certain ions. The normal dog has been found to be more satisfactory for such studies because the diet and activities can be more conveniently controlled. Further work indicated that the total base excreted does not change materially, but that there is a change in the proportion of the individual ions. It has also been shown that repeated intravenous injections over a period of several weeks brings about a condition in which there is little or no response.

## METHODS

*Dog.* Eighteen normal adult female dogs weighing 6 to 13 kg. were used. They were fed a constant diet consisting of a mixture of beef heart, Purina chow, and 2 gm. of added sodium chloride once daily. The dogs were fed at 11 p.m. daily, which was 9 hours before the beginning of a test period. This is important because, if fed too long before, the excess electrolytes are largely eliminated before the test period, so that little material remains upon which to show the effects of retention. Only those animals were selected that ingested their food within a few minutes. They were allowed as much water as they desired, except on test days. During the tests the animals were kept in metabolism cages, and at 3-hour intervals were catheterized and given 100 cc. of water by stomach tube. Urine was preserved with toluol. The dogs were kept on the standard diet until the weight became constant, and controls indicated that the animals were in balance. They were kept at about 23°C. during the whole time of the study. All injections were intravenous in order to obtain the maximum effect in the shortest time.

Extract used in these studies was prepared from dissected adrenal cortex by extracting with alcohol, passing through ether, then water, and again ether. This residue was finally taken up in water (*type 1*). One aqueous extract prepared according to this method formed a precipitate on thawing after exposure to —20°C. for days. This precipitate was dissolved in 95 per cent alcohol (*type 2*). Before injection, the alcohol solution was diluted with 9 parts of water. For some experiments further purification was obtained by starting with the final aqueous solution just described, extracting twice with an equal volume of carbon tetrachloride, then twice with one-half volume of ether, and finally

[1] Aided by a grant from The Rockefeller Foundation.

5 times with equal volumes of chloroform. The chloroform fraction was taken up in water and used after distilling off the chloroform (*type 3*). The volume of extract injected never exceeded 5 cc. and was usually less than 2 cc. With the exception of the alcoholic extract only distilled water was used. Therefore, no injections were made in control experiments. All of the extracts were sterilized by passing through a Seitz filter.

*Man.* The effect of single intravenous injections of cortical extracts was studied on 6 normal human beings. Three were on a constant diet including the addition of 2 gm. sodium chloride. The water intake was also constant. They maintained fairly uniform activity throughout the 2 weeks of observa-

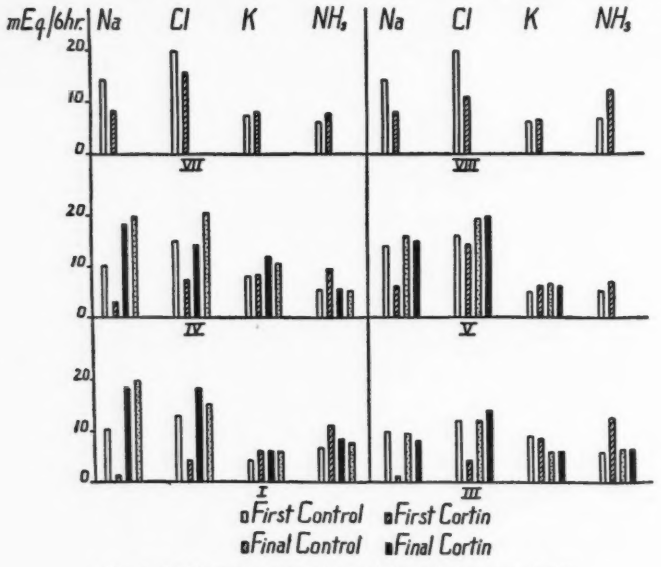


Fig. 1. Initial response in 6 dogs and the refractory state in the 4 which were injected repeatedly.

tion. Extracts (*type 1*) containing from 5 to 50 C.U. of cortin were injected at 7 a.m. on the day of the test. The last meal had been ingested at 6 p.m. on the previous evening. No food was eaten during the experimental period. Water (75 cc.) was ingested at the beginning of each hour.

The analytical methods employed were as follows: sodium by the method of Butler and Tuthill (2); potassium by the method of Hubbard (3, 4); chloride by the modified Volhard-Harvey titration method (5); ammonia by Sobel, Yuska and Cohen's modification of Van Slyke and Cullen's method (6); total base by the method of Keys (7); pH with the glass electrode.

## RESULTS

*Dog.* Initial responses are different from those obtained after repeated injections. Therefore, the two will be treated separately. Decreases in sodium ranged from 42 to 89 per cent (fig. 1). Decreases in chloride ranged from

10 to 58 per cent. Potassium was increased in all but one animal in which there was nearly a 5 per cent decrease. The increases ran as high as 22 per cent. The increase in ammonia ranged from 23 to 110 per cent.

The same extract seemed to produce a consistent sodium response in different dogs. For example, a *type 1* extract gave sodium reductions of 86, 89 and 89 per cent in 3 different dogs. *Type 1* extracts gave a more marked decrease in sodium excretion than did extracts *type 2* or *3*. The *type 2* and *3* extracts

TABLE 1. EXCRETION IN DOG XIX (8 KG.) FOR 6-HOUR PERIOD

| Treatment | Vol. cc. | Na m. eq. | Cl m.eq. | K m.eq. | $NH_3$ m.eq. | Total Base m.eq. | pH |
|---|---|---|---|---|---|---|---|
| Control | 215 | 10.24 | 14.99 | 8.24 | 7.4 | 32.0 | 6.2 |
| Control | 266 | 12.92 | 24.30 | 7.54 | 8.0 | 30.4 | 5.9 |
| Control | 390 | 13.80 | 24.00 | 7.15 | 6.2 | 29.3 | 5.7 |
| Extract *Type 3* | 130 | 6.80 | 11.55 | 8.60 | 13.0 | 29.38 | — |
| Extract *Type 2* | 216 | 5.95 | 15.60 | 7.00 | 13.9 | 30.53 | 5.8 |

produced a smaller decrease in chloride and in volume than did *type 1* extract. Here also the reductions were not far apart, being 55, 52 and 49 per cent, respectively, in 3 animals. Electrolyte changes seemed to be independent of volume (table 1).

Total base and pH determinations were made on 4 of the above 11 animals. A typical series of values is shown in table 1. The pH values following cortin injection did not change beyond the control range. Total base values showed

TABLE 2

| Dog | Weight kg. | Treatment | Total Base (Range) m.eq. per 6 hr. | pH (Range) |
|---|---|---|---|---|
| IX | 8.3 | Controls (3) | (16.72–21.61) | (5.8–6.0) |
| | | *Type 3* extract | 18.90 | 6.0 |
| XVI | 7 | Controls (6) | (23.00–30.60) | (5.6–6.0) |
| | | *Type 3* extract | 21.32 | 5.7 |
| XIX | 8 | Controls (4) | (29.30–32.00) | (5.7–6.2) |
| | | *Type 2* extract | 30.53 | 5.8 |
| XXII | 10.2 | Controls (2) | (30.76–36.00) | (5.9–6.1) |
| | | *Type 3* extract | 30.00 | 6.1 |

little or no change after the injection of extract. Whichever basic ion or ions (sodium, ammonia, potassium) are primarily affected, there are apparently compensatory changes in the amounts of the other bases excreted, so that a fairly constant level of total base is maintained (table 2).

*Effect of repeated injections.* Use of the same dog for repeated injections led us (8) to discover that the response diminished until it disappeared altogether (fig. 2). Four dogs were injected repeatedly, and all became refractory (fig. 1). On the excretion of all of the basic ions determined, the effect of

extracts decreased simultaneously. The complete refractory state required several injections and several weeks for development (table 3).

We attempted to get an indication of the duration of the refractory state by testing 3 dogs. The first was tried 2 weeks after the last injection during the refractory state, without a response to *type 1* extract. The second was tried 11 weeks after the last injection during the refractory state with a 27 per cent reduction in sodium from a *type 1* extract. (This extract gave 86 per cent reduction following the initial injection in this dog.) The third was tried 12 weeks after the last injection during the refractory state with a 37 per cent reduction in sodium following a *type 3* extract. (This extract gave an initial response of 42 to 55 per cent decrease in 5 other dogs.)

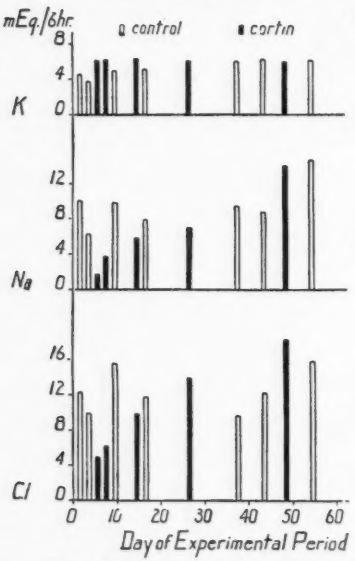


Fig. 2. Development of the refractory state from repeated injections of cortin (20 C.U.) in a dog weighing 13 kg.

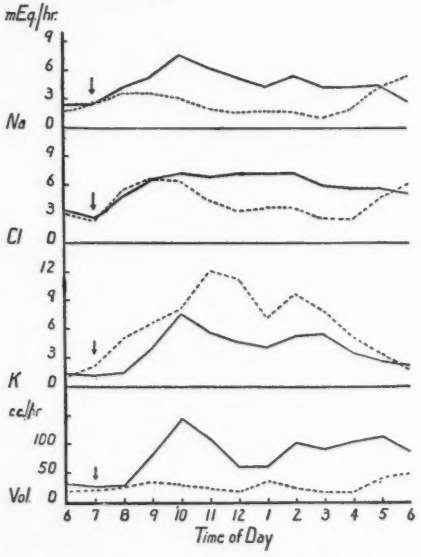


Fig. 3. Duration of electrolyte effects from the injection of 15 C.U. of cortin in a human being.
Solid line = control.
Broken line = cortin.

The urine of all dogs was tested for albumin and examined microscopically at frequent intervals. The findings were always normal. Urea excretion during the experimental period remained the same in all animals. No animals showed any ill effects from the experiments. One dog (III) was killed 2½ months after the beginning of the experiment. Histological examination showed that the thyroid, parathyroids, liver, spleen, adrenal and kidney were normal.

Some indication of the nature of the refractory state has been obtained in experiments to be reported elsewhere. The serum of refractory dogs, I and III, injected intravenously just prior to the cortical extract, inhibited the electrolyte response. Extracts (*type 1* and *3*) which gave good responses in untreated dogs were used in the usual dosage 20 to 40 C.U. These sera were injected into 5 dogs; all failed to give the electrolyte response to cortical extract. This trans-

ferred refractory state seems to be temporary. Twenty-four days after receiving injections of serum and extract, one dog (XVI) was given 40 C.U. of *type 3* extract intravenously. This resulted in a 50 per cent decrease in sodium, a 40 per cent increase in ammonia, and a 4 per cent increase in potassium.

*Man.* The results on the human being were similar to those obtained on the dog. A typical experiment is shown in figure 3. Changes in electrolyte excretion (retention of sodium and release of potassium) appeared within an hour. The chloride retention, which was not as marked as that for sodium,

TABLE 3. FACTORS IN THE DEVELOPMENT OF THE REFRACTORY STATE

| Dog | Wt. kg. | Initial Na decrease % | No. of injections | C.U. Per injection | Days treated | Time when complete refractory state was first observed | Remarks |
|---|---|---|---|---|---|---|---|
| I | 13 | 86<br>With 20 C.U. *type 1* Ext. | 4 | 20 | 21 | At 6 weeks | 3 more injections made after development of the refractory state.<br>11 weeks after last injection tested with 20 C.U. Ext. *1* 27% decrease in Na. |
| III | 8.5 | 89<br>With 20 C.U. *type 1* Ext. | 5 | 20 | 28 | At 5 weeks | 6 more injections made after development of the refractory state.<br>2 weeks after last injection tested with 20 C.U. Ext. *1* No response. |
| IV | 7 | 66<br>With 40 C.U. *type 1* Ext. | 4 | ?<br>40<br>40<br>60 | 10 | At 2 weeks | 3 months after final injection tested with 40 C.U. Ext. *3*. 37% drop in Na. |
| V | 6 | 47<br>With 30 C.U. *type 3* Ext. | 4 | ?<br>?<br>30<br>30 | 21 | 5 weeks after first injection giving electrolyte response | |

might be later in appearing. The maximum effect from a single injection was reached in 4 to 6 hours. In about 10 hours the effect disappeared. The sodium retention for the 6 subjects from the injection of 10 to 20 C.U. of cortin ranged from 33 to 65 per cent. It varied in different individuals for the same dose; e.g., in 3 different subjects with 20 C.U. it was 41.5, 51 and 65 per cent respectively.

The refractory state also seems to develop in the normal human being. One subject who responded by reductions of 52.7 and 17.6 per cent in the sodium excreted after the intravenous injection of 15 and 5 C.U. respectively, failed to respond to 40 C.U. 2 months later. There had been 5 injections of extract during the intervening period.

## DISCUSSION

The duration of sodium retention after cortin injection indicates that the effect disappears in 10 hours or less. If this is true in adrenal insufficiency, 3 injections in 24 hours are required for adequate treatment. In normal subjects, injections are not required and therefore may not be used up so quickly as in adrenal insufficiency. However, we do know that the same amount of hormone is more effective in divided doses than as a single dose. Even greater frequency of injection is required in stress. The effect of intravenous injection on electrolytes also indicates that cortin is not quickly destroyed.

Since our work was completed, a paper by Harrop and Thorn (9) has appeared. They have given 1000 D.U. of cortin intravenously with 55 per cent reduction in sodium excretion for a 6-hour period. This is similar to our results except that the dose is very much larger, perhaps as much as 30 times. With the exception of this one experiment, all of their injections were given subcutaneously in 2 doses over a 24-hour period. Their results with subcutaneous injection were not greatly different, except that the potassium excretion was much larger than ours. They have suggested the electrolyte effect as a method of assay, stating that "the results obtained on any given animal are reproducible." We had started with the same idea, but the development of the refractory state made that impossible. It is difficult to understand why they did not observe the development of the refractory state. The doses injected were certainly large enough, and although given subcutaneously the absorption was rapid enough to produce electrolyte effects of a magnitude similar to ours. The number of injections and duration of experiments with the same animal are not reported in their paper.

## SUMMARY

The effect of intravenous injections (20 to 40 C.U.) of cortin on the renal excretion of electrolytes was studied on 18 normal dogs. The initial response ranged as follows: sodium retention, 42 to 89 per cent; chloride retention, 10 to 58 per cent; potassium, from a 5 per cent decrease to a 22 per cent increase; the ammonia increase ranged from 23 to 110 per cent. Electrolyte changes seem to be independent of urine volume. Urine pH did not change beyond the control range. Total base showed little change, the retention of sodium and the release of potassium and ammonia are always associated. Repeated injections in the same dog led to the development of a refractory state in which there was no response. This required several injections and several weeks for development. All four dogs that were tried became refractory. The refractory state lasted for weeks, showing partial disappearance in 3 months. It can be transferred to a new dog by injection of serum from a refractory dog, but the transferred refractory state seems to be temporary.

Similar results were obtained in the study of 6 normal human beings, in whom the retention of sodium lasted for about 10 hours after a single intravenous injection of 5 to 50 C.U. of cortin. The refractory state developed in the one subject who was injected repeatedly.

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# MINERAL METABOLISM OF ADRENALECTOMIZED RATS STUDIED BY THE APPETITE METHOD

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Our knowledge of the mineral metabolism of adrenalectomized animals is based almost exclusively on studies made on the mineral content of the blood, bones, urine and feces. In the present paper it will be shown that additional knowledge may be obtained from an entirely different source, viz., the animal's mineral appetite or craving. In preliminary experiments it was found that mineral losses are closely reflected by an increased mineral appetite. Adrenalectomized rats show an increased appetite for sodium chloride, and parathyroidectomized rats an increased appetite for calcium lactate. Because of these increased appetites, the animals survived and were able to keep themselves free from symptoms of insufficiency and tetany (1, 2).

This appetite method has now been used to study in greater detail the changes produced by adrenalectomy in mineral metabolism. The following study was made to determine whether adrenalectomy would produce an increase in appetite for sodium salts other than sodium chloride, and whether it would produce an increased appetite for minerals other than sodium.

## METHODS

The two types of cages used in these experiments are shown in figure 1. The first type used for single mineral choice experiments was 7¾ in. wide, 12½ in. long, and 10 in. high, and contained besides a food cup 2 graduated inverted water bottles, one for tap water, and another for a mineral solution. The other type used for multiple mineral choice experiments was 30 in. long, 12½ in. wide, and 9 in. high, and was divided into 3 compartments, one for each rat. Each compartment contained 7 bottles, one for tap water and 6 for different mineral solutions.

The determination of a working concentration of the mineral solution to be offered the animals is of prime importance in these appetite experiments. On the one hand, the solution must be sufficiently concentrated so that the animals can distinguish it easily from water, and obtain the desired amount of a mineral without increasing their water intake; on the other hand, it must not be so strong as to be unpalatable.

The concentration of each mineral solution was arrived at empirically by the following technique. Six normal adult animals were placed in separate cages of the same type shown in figure 1 *A*, but provided with only one bottle containing tap water. After a fairly constant level of fluid intake was es-

tablished, usually within 10 days, the water bottle was removed and replaced with a bottle containing the mineral solution to be standardized, and in a concentration that was just palatable (as judged by members of the laboratory). Thus the animals were forced to drink the mineral solution in order to satisfy their thirst. If their fluid intake continued unchanged, it was concluded that the concentration was too low and had to be increased; if it showed an immediate sharp drop, it was concluded that the concentration was too strong and had to be decreased. In either case, the concentrations were changed every 4 to 6 days, until the fluid intake was about one third lower than with water alone. Finally the water bottles were put back on the cages, giving the rats a choice of water and the mineral solution. If they still drank a small amount (about 2 to 5 cc.) of the mineral solution every day, in addition to the usual

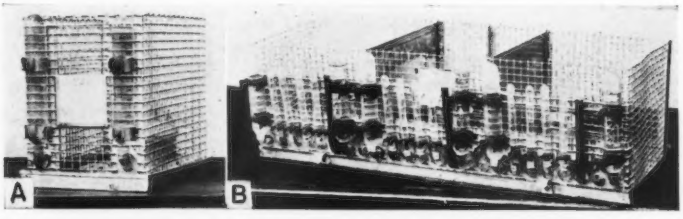


Fig. 1. Cages used: *A*, for single mineral choice experiments; *B*, for multiple mineral choice experiments.

amount of water intake, it was decided that a satisfactory concentration had been obtained.

About 40 different mineral solutions have been standardized in the above manner during the past year. The concentrations of the various solutions used in these experiments were as follows.

| | | | |
|---|---|---|---|
| Sodium chloride | 3.0% and 1.0% | Ammonium chloride | 1.0% |
| Sodium lactate | 4.0% | Ammonium sulphate | 1.0% |
| Dibasic sodium phosphate | 2.0% and 4.0% | Ferric chloride | 1.0% |
| Sodium iodide | 1.0% | Aluminum chloride | 1.0% |
| Potassium chloride | 2.0% and 3.0% | Ferrous chloride | 1.0% |
| Calcium chloride | 1.0% and 2.0% | Calcium lactate | 2.4% |
| Magnesium chloride | 1.0% | | |

In these experiments the salt content of the food was also of great importance. Three different food mixtures were used, the standard McCollum diet which contained 1 per cent sodium chloride; the same diet without sodium chloride; Cox's low mineral diet (3). On the standard McCollum diet, the animals received part of their salt from the food and part from the mineral solution while on the same diet without salt, and on Cox's low mineral diet practically all of the salt was obtained from the voluntarily ingested mineral solutions.

The fluid intake was recorded daily at noon. Body weight was recorded weekly. The bottles were thoroughly cleaned and filled usually every third day. The animals were placed in the cages at an average age of 80 days, and records taken for several weeks, at least until constant base lines for the intake of the water and test solutions were obtained. Then the adrenalectomy was per-

formed. Records were taken usually for 40 days afterwards, in some instances for much longer periods.

It is sufficient to say that in previous adrenalectomy experiments on 26 rats, of approximately the same age and fed on the McCollum diet, the mortality was 61 per cent. For 12 animals on a salt free McCollum diet the mortality was 100 per cent.

When the animals died or were killed, all of the endocrine glands were

TABLE 1

| Solution | No. of rats | Age at adrenalectomy, days | Per cent mortality | Average daily salt intake in cc. | | Average daily sodium intake in gm. | | Average daily chloride intake in gm. | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | 10-day period before adrenalectomy | last 10 days of life | 10-day period before adrenalectomy | last 10 days of life | 10 day period before adrenalectomy | last 10 days of life |
| *A (sodium solutions)* | | | | | | | | | |
| | | | | REGULAR FOOD | | | | | |
| No choice | 26 | | 61 | | | | | | |
| 4.0% sodium phosphate | 4 | 95 | 50 | 5.5 | 14.1 | 0.03 | 0.07 | | |
| 4.0% sodium lactate | 3 | | 33 | 3.7 | 14.7 | 0.03 | 0.12 | | |
| 1.0% sodium chloride | 13 | 80 | 31 | 16.9 | 27.6 | 0.07 | 0.11 | | |
| | | | | SALTLESS FOOD | | | | | |
| No choice | 13 | 90 | 100 | | | | | | |
| 4.0% sodium lactate | 3 | | 100 | 5.3 | 6.4 | 0.04 | 0.05 | | |
| 3.0% sodium chloride | 5 | 93 | 20 | 2.2 | 12.5 | 0.03 | 0.15 | | |
| *B (chloride solutions)* | | | | | | | | | |
| | | | | REGULAR FOOD | | | | | |
| 3.0% potassium chloride | 7 | 63 | 100 | 3.0 | 2.9 | | | 0.028 | 0.027 |
| 1.0% magnesium chloride | 2 | 141 | 100 | 2.3 | 3.2 | | | 0.017 | 0.024 |
| 1.0% aluminum chloride | 2 | 126 | 100 | 2.1 | 2.8 | | | 0.016 | 0.022 |
| 1.0% ammonium chloride | 1 | 108 | 100 | 7.1 | 6.4 | | | 0.047 | 0.042 |
| | | | | SALTLESS FOOD | | | | | |
| 2.0% potassium chloride | 5 | 119 | 80 | 2.0 | 3.2 | | | 0.019 | 0.031 |
| 2.0% calcium chloride | 3 | 118 | 100 | 1.7 | 2.0 | | | 0.011 | 0.013 |
| 1.0% ferrous chloride | 2 | 138 | 100 | 2.5 | 1.6 | | | 0.014 | 0.009 |
| 1.0% ferric chloride | 1 | 134 | 100 | 2.3 | 3.0 | | | 0.015 | 0.020 |
| 1.0% ammonium chloride | 6 | 113 | 100 | 4.5 | 5.7 | | | 0.030 | 0.038 |

weighed and preserved for histological study, and a thorough search was made with a dissecting microscope for regenerated adrenal tissue, or accessory bodies.

## RESULTS

*Single choice of sodium solutions.* The intake of sodium lactate, as well as of sodium phosphate was definitely increased by adrenalectomy. Typical records are presented in figure 2, *D* and *E*, of two animals on the regular McCollum diet, one with access to sodium lactate (4 per cent), the other to sodium phosphate (4 per cent). The sodium lactate intake of the first animal increased from 2.5 cc. per day for the 10-day period before adrenalectomy to 14.1 cc. 25 days later. Adrenal implants (donor 4 days) to the anterior

chamber of the eye caused the sodium intake to return to its pre-operative level within 12 days. Body weight increased rather than decreased. At the time the animal was killed, 69 days after adrenalectomy, it was in good health and free from symptoms of insufficiency. The graft showed normal cortical tissue. No adrenal tissue was found near the kidneys.

In the other animal (fig. 2 *E*), the sodium phosphate intake increased from a level of 4.7 cc. per day for the 10-day period preceding adrenalectomy to

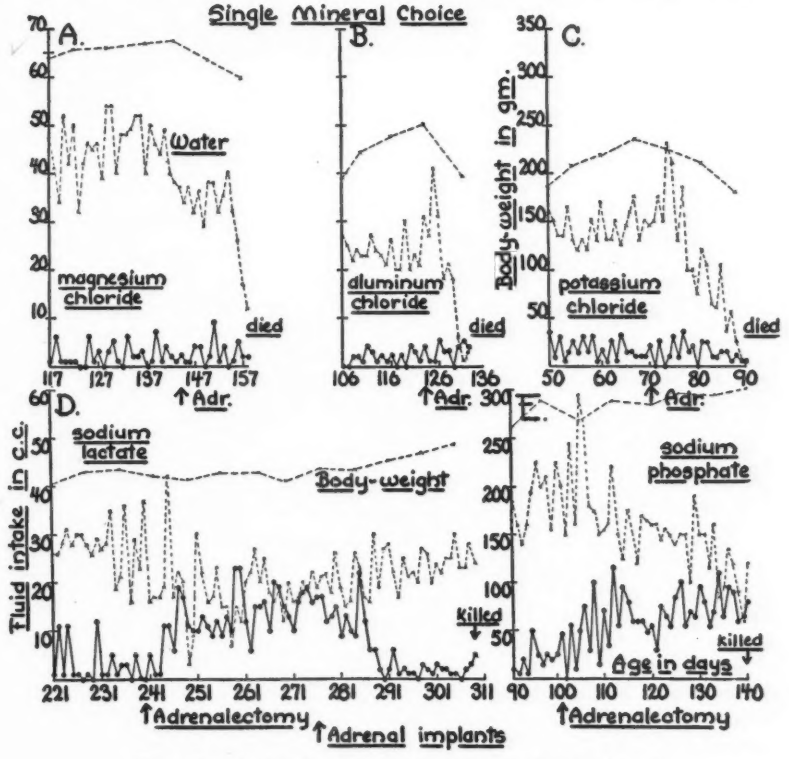


Fig. 2. Records of single mineral choice experiments showing intake of mineral solutions and of water before and after adrenalectomy.

15.6 cc. in the 30 to 39-day interval afterwards. Water intake was inversely proportional to the sodium phosphate intake, and body weight increased. When the animal was killed 39 days after adrenalectomy, no adrenal tissue was found.

The results of these experiments are summarized in table 1 *A*, which gives the average salt intake for 10 days before adrenalectomy, and for the last 10 days of life for animals on the regular McCollum diet and on the salt-less McCollum diet. On the regular diet the mortality was 61 per cent for untreated animals, 50 per cent for animals offered a sodium phosphate solution, 33 per cent for animals offered a sodium lactate solution and 31 per

cent for animals offered a 1 per cent sodium chloride solution. On the saltless diet the mortality was 100 per cent for untreated animals, 100 per cent for animals offered sodium lactate solution, and 20 per cent for those offered a 3 per cent sodium chloride solution. It will be noted that the mortality was roughly inversely proportional to the amount of the ingested sodium. Animals in which any adrenal tissue was found have been excluded.

These results show that the adrenalectomized animals had a definitely increased appetite for sodium, and that they got along best when sodium was offered with chloride.

*Single choice of chloride solutions.* The intake of chloride solutions of minerals other than sodium was not changed by adrenalectomy. Three typical records in figure 2 *A, B* and *C* are of animals having access to chlorides of

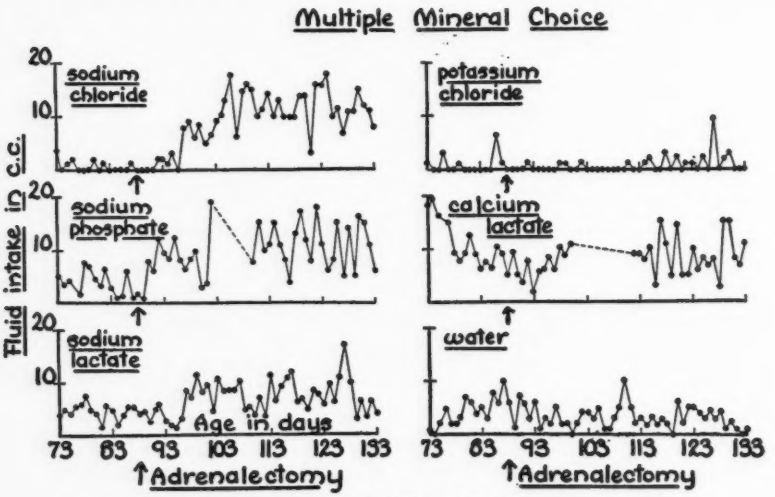


Fig. 3. Record from one animal showing effect of adrenalectomy on intake of water and 5 mineral solutions.

magnesium (1 per cent), aluminum (1 per cent), and potassium (3 per cent) respectively. The intake of the chlorides after adrenalectomy was much the same as before; body weight and water intake showed the typical decrease of untreated animals, and death occurred within 20 days.

The results are summarized in table 1*B*. On the regular food the intake of the chloride solutions was not increased in any of the groups. All of the animals died after a very short interval, and on the saltless food all of the animals died except one on potassium chloride, which animal showed a small increase in potassium chloride intake. No adrenal tissue was found at autopsy in any of these animals.

*Combined choice of sodium and chloride solutions.* Eleven animals on a low mineral food (3) were given access to tap water and 5 solutions, sodium chloride 3 per cent, sodium lactate 4 per cent, sodium phosphate 2 per cent, potassium chloride 2 per cent, and calcium lactate 2.4 per cent. These animals were kept in cages of the type shown in figure 1*B*.

A typical record of one of the animals is presented in figure 3. Adrenalectomy produced a definite increase in the intake of sodium chloride, sodium phosphate and sodium lactate. The intake of potassium chloride, and calcium lactate showed a very small increase after several weeks. Body weight increased steadily until the animal was killed 42 days after adrenalectomy. At autopsy no adrenal tissue was found.

All 11 animals gave indication of living indefinitely. Thus the voluntary ingestion of these mineral solutions changed the mortality from 100 per cent for untreated animals on the Cox's diet to 0. No other form of therapy used in our colony showed so low a mortality rate.

The results of these experiments on the 11 animals are summarized in figure 4, which shows the average daily intake of the 5 different mineral solutions for 1 ten-day period before adrenalectomy and for 4 ten-day periods afterwards. Before adrenalectomy, the intake of sodium chloride, sodium lac-

TABLE 2. COMBINED CHOICE OF SODIUM AND CHLORIDE SOLUTIONS BY ADRENALECTOMIZED RATS (11 animals)

| | Average daily intake of mineral solutions in cc. | | Average daily sodium intake in gm. | |
|---|---|---|---|---|
| | 10-day period before adrenalectomy | 30–40 day period after adrenalectomy | 10-day period before adrenalectomy | 30–40 day period after adrenalectomy |
| 2.4% calcium lactate | 8.91 | 8.32 | | |
| 3.0% sodium chloride | 1.31 | 8.40 | 0.015 | 0.100 |
| 2.0% sodium phosphate | 6.10 | 7.65 | 0.016 | 0.019 |
| 4.0% sodium lactate | 1.83 | 4.37 | 0.014 | 0.035 |
| 2.0% potassium chloride | 0.43 | 0.79 | | |
| Total sodium intake: | | | 0.045 | 0.154 |

tate and potassium chloride was low, while the intake of calcium lactate and sodium phosphate was high. These differences undoubtedly reflect unequal deficiencies created by the low mineral diet.

After adrenalectomy, sodium chloride intake showed a great increase; sodium lactate also increased, but less markedly, and potassium chloride showed only a small increase. The fact that even before adrenalectomy most of the animals showed a very high intake of the calcium lactate and sodium phosphate solutions makes it difficult to say whether the intake of these solutions was increased. However, several individual records did show a definite increase in both solutions.

These results are summarized in table 2. Most of the sodium was obtained from the sodium chloride, 0.100 gm. compared with 0.019 and 0.035 gm. for sodium phosphate and sodium lactate respectively. The total daily sodium intake was 0.154 gm.

In order to overcome the great differences in intake of the different solutions before adrenalectomy it was decided to keep the animals on the regular McCollum diet instead of the low mineral diet. Wherefore it could be assumed that an adequate amount of all of the minerals would be received. Furthermore, it was decided to offer a selection of solutions containing most

of the electrolytes ordinarily present in blood plasma. The following solutions were used: sodium chloride (3 per cent), sodium phosphate (2 per cent), potassium chloride (2 per cent), ammonium sulphate (1 per cent), calcium lactate (2.4 per cent), and sodium iodide (1 per cent).

As was expected, before adrenalectomy these animals on the regular McCollum diet took a much smaller amount of the minerals than was taken by those on the low mineral diet. Nevertheless, they all took a small amount of each solution, most of sodium chloride and sodium phosphate and least of calcium lactate and potassium chloride. Judging from their appetite for these mineral solutions, it appeared then that the amount of calcium and potassium supplied by the McCollum diet was adequate, but that the amount of sodium and phosphate was slightly inadequate.

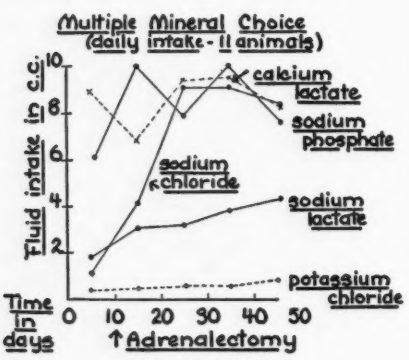


Fig. 4

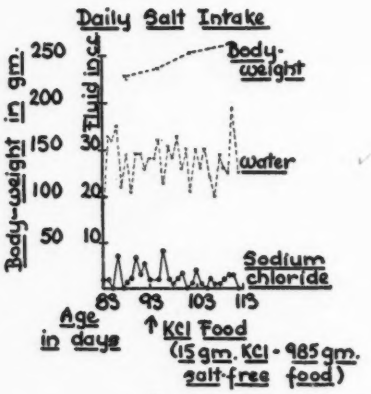


Fig. 5

Fig. 4. Effect produced by adrenalectomy in 11 animals on average daily intake of 5 different mineral solutions.
Fig. 5. One animal showing slight decrease in salt appetite produced by high potassium diet.

After adrenalectomy, the animals immediately began to take more of all of the solutions except sodium iodide. The daily intake showed a continued and steady increase throughout the 40-day period. Sodium chloride showed the greatest increase, potassium chloride the least. All of the animals gained weight at approximately the normal rate and gave every indication of living indefinitely.

The results for the 6 animals are summarized in figure 6, which shows the average total daily intake of the different ions for 10 days before adrenalectomy, and for the following 3 ten-day periods. In the 20 to 30-day period, the intake of sodium and chloride was highest, 137 and 163 mg. respectively; the intake of calcium and potassium was lowest, 15 and 17 mg. respectively; and the intake of lactate and phosphate fell midway between, with 58 and 87 mg. The fact that the intake of these different ions was still increasing at the end of 30 days must indicate that they were being lost from the body at an increasing rate at this time. The flattening off of the curves within the

next 20 days at this high level probably indicated that a state of equilibrium had been reached.

At the end of 60 days, the animals were injected daily with 0.5 cc. of cortical extract (eschatin).[1] The intake of the different mineral solutions returned approximately to their original levels within 15 days. The most rapid and marked response to the injections was shown by the sodium solutions, particularly sodium chloride. No adrenal tissue was found at autopsy.

It is of special interest that the relative proportions of the different ions ingested are essentially the same as are found in normal human blood serum. The graph at the right in figure 6 shows the weights of each of these different ions in normal blood serum from humans. Here again, sodium and chloride are highest, potassium and calcium lowest, the lactate and phosphate midway between.

It can be concluded from these experiments that adrenalectomy produces a loss of all these ions, rather than sodium alone. Similar conclusions were arrived at by Rubin and Krick (4) on the basis of metabolic studies made on urine and stools of adrenalectomized rats. They reported a loss of sodium, chloride, magnesium, calcium and potassium. This conclusion is not in agreement with reports of a potassium retention rather than loss (5, 6, 7), although several other recent works based on urine and blood analysis have also reported a potassium loss (4, 8).

The potassium-sodium relationship in adrenalectomized animals deserves some further comment. It has been claimed that adrenalectomized animals do not survive when the potassium intake is increased. Our experiments have shown that when the intake of sodium and other blood plasma ions is also increased, the potassium intake may actually be greatly increased above the normal level with definitely beneficial results.

The inverse relationship between sodium and potassium so generally accepted ever since Bunge (9) in 1873 announced his theory that the increased potassium intake is the basis for the salt appetite of herbivorous animals may also be subject to revision. Bunge traced the increased salt appetite of herbivorous animals to the fact that the content of the herbivorous diet was high in potassium rather than low in salt, and the excess of potassium was very readily excreted in the urine, carrying sodium with it in large quantities because of some special relationship between these ions. We have tested this theory with the following experiment. Eight rats on a saltless McCollum diet were given a choice of water and a 3 per cent salt solution. Daily records were taken for several weeks, until fairly constant levels of salt intake were attained. Then large amounts of potassium chloride were mixed with the food (15 gm. per 1000 gm. of food for the first 4 weeks, later 45 gm.). Not one of the animals showed an increased salt solution intake; in fact, 3 showed a definite decrease. The record of one of the animals is shown in figure 5. The observations were carried on for 60 days, and we feel that they definitely disprove Bunge's theory. Furthermore, they add evidence that in adrenalec-

[1] Kindly supplied by Parke, Davis & Company.

tomized animals the increased intake of the sodium ion as well as of the potassium ion is due directly to a loss of each of these ions, rather than to any inverse relationship between them.

*Latency period of the onset of the increase in sodium appetite.* In the 38 animals, the interval between adrenalectomy and the onset of the increase in sodium appetite varied from 1 to 9 days, and averaged 3.3 days. Some animals showed the increased sodium appetite almost at once, i.e., within 24 hours or less after adrenalectomy, and reached their maximum intake within the first 4 days. Others gave no indication of any increase until 5 to 9 days after the operation, and in several instances did not reach a maximum intake for 20 days or more.

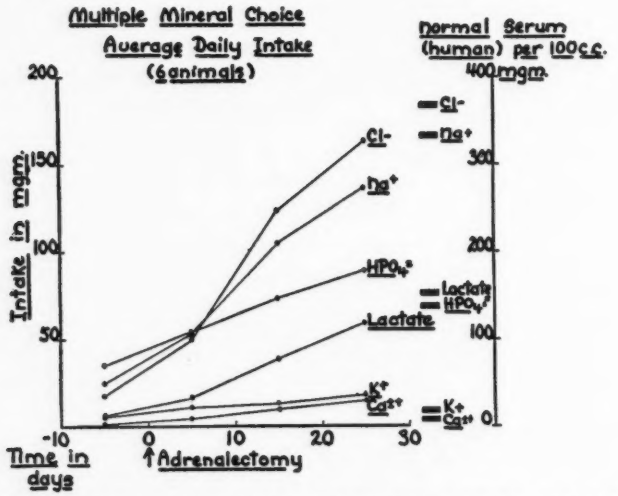


Fig. 6. Effects produced by adrenalectomy in 6 animals on average daily intake of 6 different ions; also amount of each of these ions present in normal blood serum.

## DISCUSSION

The fact that the adrenalectomized rats made advantageous selections from the various chemical solutions offered brings further evidence for the validity of Cannon's concept of the 'wisdom of the body' (10).

It is not possible at present to state whether the ability of the rat to make beneficial selections of minerals depends on experience or on more deeply lying biological factors concerned with chemical changes in the taste and smell mechanisms. It may be that the animal learns to associate relief from insufficiency symptoms with the ingestion of certain minerals and not with others; or it is possible that glandular disturbances produce chemical changes, particularly in the taste apparatus, which result in making certain minerals much more desirable or pleasant, quite independently of any experience.

An important point to call to the attention is the fact that, although on this mineral diet the adrenalectomized rats showed normal growth and free-

dom from symptoms of insufficiency, according to our activity criterion they were not really fully restored to their normal state. Measurements of activity (in revolving drums) showed that they were all very inactive. In a previous paper it was contended that claims of full adrenal replacement are justified only when the animals are made normally active again (1). So far, this result has been achieved only by transplants of adrenals. Injections of cortical extract and feeding of Grollman's charcoal adsorbate have produced only partial replacement. Obviously some other and very important factor is still lacking in the mineral as well as in the cortical extract therapy. It may be concerned with control of the fatigableness of the muscles (11). It is possible, however, that activity replacement might have been achieved had the rats been offered a still wider selection of minerals.

## SUMMARY

The mineral metabolism of adrenalectomized rats was studied by the appetite or self-selection method. Adrenalectomy produced a definitely increased appetite (10-fold in some instances) for sodium lactate (3 animals) and sodium phosphate (4 animals) solutions, as well as for sodium chloride (13 animals). In several animals implants of adrenal tissue to the eye caused the appetite to return to its normal level. In general, the mortality varied inversely with the intake of sodium.

In the single choice experiments, adrenalectomy did not increase the appetite for chlorides of iron, magnesium, calcium, aluminum, potassium or ammonium. In the multiple choice experiment, 11 rats on a low mineral diet were given a choice of sodium chloride, sodium lactate, sodium phosphate, potassium chloride, calcium lactate and water. Adrenalectomy produced an increased intake of the sodium solutions, but also a small increase in potassium chloride. The mortality was reduced from 100 per cent to 0. In a second multiple choice series, 6 rats on regular McCollum diet were given a choice of a wider assortment of electrolytes, sodium chloride, sodium phosphate, sodium iodide, potassium chloride, ammonium sulphate, calcium lactate, and water. Adrenalectomy produced an increased appetite for all solutions except sodium iodide. The relative proportions of the increased intake of the different ions were practically the same as are found in normal blood serum. Sodium and chloride ions showed the greatest increase, potassium and calcium ions the smallest, and the lactate and phosphate ions fell midway between.

Daily injections of cortical extract (0.5 cc. eschatin) caused the mineral appetite of these 6 animals to return approximately to its normal level. The latency period of the increase in sodium appetite averaged 3.3 days, with a range of variation from 1 to 9 days.

It was concluded that adrenals regulate not only the metabolism of sodium, but of most of the other electrolytes found in normal blood serum.

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# CALCIUM AND PHOSPHORUS STUDIES

## XIV. THE EFFECT OF REPEATED DOSES OF PARATHYROID EXTRACT ON THE CHEMICAL COMPOSITION OF THE BLOOD AND URINE OF THE DOG: AN EXPLANATION OF THE CAUSE OF DEATH IN PARATHYROID OVERDOSAGE

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Soon after the isolation from beef parathyroids of an active principle, capable of raising the levels of calcium in the serum of normal and parathyroprivic dogs (1), Collip (2) and others (3, 4, 5, 6) found that repeated, moderately large doses of the extract were extremely toxic. They observed that the injection into dogs of about 125 I.U. of parathyroid extract at frequent intervals produced a fairly rapid rise in the levels of serum calcium, which reached a concentration of about 20 mg. per cent in 20 hours and remained at this level for some time. However, in spite of the administration of the extract, the serum calcium concentration usually declined, and the inorganic phosphorus increased, in about 36 hours. The increased excretion of calcium and phosphorus in the urine, which began shortly after the injections were started, then declined sharply as though the injections had been interrupted. The concentrations of urea and of nonprotein nitrogen in the blood increased steadily, and were followed later by oliguria or anuria. Anorexia, vomiting, diarrhea, and lassitude were followed by muscular atony, uremic coma, and death. Shortly before death, it became difficult to draw blood and to separate the serum from the clot. All the signs and symptoms of anhydremia and failing circulation were present: increased cell count, decreased blood volume, and a weak, thready, and irregular pulse. Postmortem examination usually revealed congestion and hyperemia of the viscera, and the presence of blood in the stomach and intestines. Metastatic calcification was demonstrated grossly and histologically, especially in Bowman's capsule and the tubules of the kidney, in the wall of the stomach, in the walls of the bronchi and alveoli of the lungs, in the interstitial connective tissue and muscle of the heart, and in the medial coats of the lesser arteries.

These symptoms of acute overdosage with parathyroid extract are generally attributed to renal damage, since there is usually anuria or oliguria, retention of nonprotein nitrogen in the blood, and often also lime-salt deposition in the kidneys. This suggestion, however, does not explain why acute parathyroid 'poisoning' occurs but rarely in human beings suffering from osteitis fibrosa cystica and exhibiting constant high calcium levels in the blood, or why the same substance, parathyroid extract,

which is known to act as a diuretic (7, 8, 9, 10, 11), suddenly reverses its activity and prevents not only diuresis but even the excretion of normal amounts of urine. It occurred to us that the preterminal urinary supression and uremia might be explained on the basis of loss of electrolytes and water during the periods of diuresis. This is suggested by the fact that parathyroid extract acts as a diuretic in tetany and in cardiac and nephritic edema, and also from the fact that such a diuresis is accompanied by an increased excretion of electrolytes other than calcium and phosphorus. Thus, Albright and Ellsworth (11) obtained more than a 100 per cent increase in the excretion of water and chlorides after the administration of parathyroid extract to a boy suffering from idiopathic tetany, and McCann (8) noted more than a 700 per cent increase in the concentration of urinary chlorides accompanying the parathyroid extract diuresis in nephritics. A similar effect was noted by Albright, Bauer and Aub (12) in subjects injected with the extract, namely, an increased urinary excretion of water, chlorides and total base. More recently, Bassett and van Alstine (13) demonstrated that parathyroidectomy for the relief of osteitis fibrosa produces the opposite effect, namely, a retention of Na, K, Mg, Cl and water, in addition to the lime salt retention.

With these facts in mind, the sequence of events in parathyroid extract overdosage may be visualized as follows. In the production of parathyroid extract overdosage, after the animal receives a moderate dose of the extract diuresis follows, and before the animal recovers from the effect of the first injection, another dose is administered, which also produces a diuresis. If the 4-hourly injections are continued for 24 to 48 hours, less and less urine is excreted until anuria results. It must be quite evident that if the diuresis so produced is associated with an increased excretion of electrolytes other than calcium phosphate, and if the animal receives neither food nor water during the experimental period, then the end results (dehydration, hypochloremia, hyperphosphatemia, nonprotein nitrogen retention, anuria, uremia, and death) are similar to those resulting from vomiting, diarrhea, or intestinal fistulae. The experiments described in this paper were planned to prove or disprove this thesis, and, if confirmatory, to study the effect of the administration of solutions of electrolytes or of glucose as prophylactic and therapeutic measures against the ill effects of overdosage with parathyroid extract.

## EXPERIMENTAL PROCEDURE

Male dogs were used in all the experiments. With the exception of one experiment in which puppies were used, the animals employed were fairly large-sized adults. They were kept in the kennel for several days before each experiment, and trained in the laboratory to submit to venepuncture and clysis and to catheterization. They were fed the regular kennel ration, consisting of cooked beef hearts and lungs, supplemented by hospital scraps of bread, beef and bones. Food was withheld for about 18 hours prior to the experiment, and food and water were withheld during the entire period of the study, unless given as a therapeutic measure. Before the experiment was begun, blood was drawn from an anterior leg vein for the determination of the following constituents: calcium, inorganic phosphorus, chloride, sodium, nonprotein nitrogen, carbon dioxide combining power, and, in many instances, also for hemo-

globin and total serum protein (refractometric). A 4-hour specimen of urine was obtained in each case by catheterization just before the first injection of the extract.[1] The urines so collected were measured and subsequently analyzed for the following constituents: calcium, phosphorus, sodium, chloride, total nitrogen, and in one instance also for potassium. The parathyroid extract was injected at 4-hourly intervals with from 6 to 13 injections, the dosages varying with the size of the animal and with the purpose of the experiment. Samples of blood were usually drawn at intervals of 12 to 24 hours, and, in some cases, oftener. Urines were obtained by catheterization at intervals of 3 to 8 hours. The urinary volume and the urinary constituents, shown in the figures, are calculated in terms of hourly excretions.

The procedure in the treated animals was similar to the above, except that 200 cc. of either salt solution of 6.0 per cent glucose solution were admin-

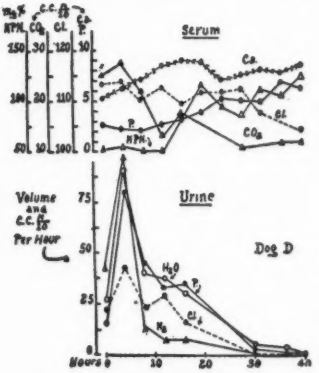


Fig. 1

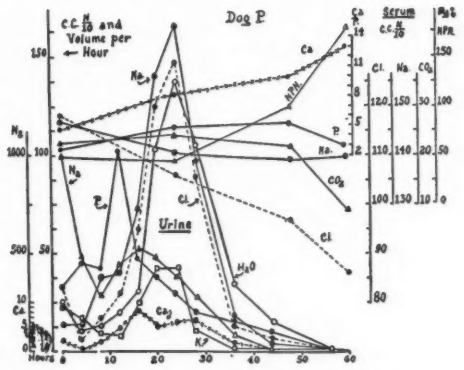


Fig. 2

Fig. 1. Effect of repeated large doses of parathyroid extract on the chemical composition of the blood and the excretion of water, chlorides, phosphorus, and nitrogen in dog *D*. Diuresis began almost immediately after the first injection, and the phosphate excretion did not precede the water and chloride excretion. (Ca, Na, and K in the urine were not determined in this animal.)

Fig. 2. Effect of repeated doses of parathyroid extract on the chemical composition of the blood and the excretion of water, nitrogen, and electrolytes in dog *P*.

istered intravenously at 4-hourly intervals, at a rate of about 10 cc. per minute. All blood samples were obtained in chemically clean syringes before therapy was begun.

In case of death, the animals were autopsied as soon after exitus as possible so as to prevent tissue autolysis. Gross inspection was made of all the important viscera, but particular attention was paid to the presence or absence of metastatic calcification in the kidneys and heart muscle. Where calcification appeared to be absent in the kidneys grossly, the findings were checked by histological sections.

## RESULTS

*Effect of repeated injections of parathyroid extract.* In this part of the experiment, dealing with the effect of the extract on the chemical composition of the blood and urine, 6 dogs were used. Examples of the results obtained in 2

[1] The parathyroid extract was generously supplied by Eli Lilly Co., Indianapolis, Indiana.

such experiments are shown in figures 1 and 2. Figure 2 shows the chemical changes in dog *P.* This animal weighed 30 kg. and received 12 four-hourly injections of 100 U of parathyroid extract (3.3 U per kg.), or a total of 1200 U. The urinary excretion of water in the fore-period averaged 25 cc. per hour; at the 16th hour after the initial injection, diuresis began and reached a maximum of 138 cc. per hour at the 24th hour. The diuresis, however, declined quite rapidly to below normal values at the 36th hour, and urine formation ceased completely a few hours before exitus. The excretion of sodium and chloride, expressed in cc. N/10 per hour, followed closely the trend of water excretion, so that the percentages of these substances in the urine were ap-

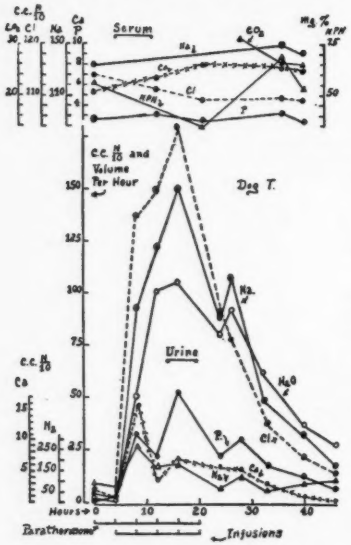


Fig. 3

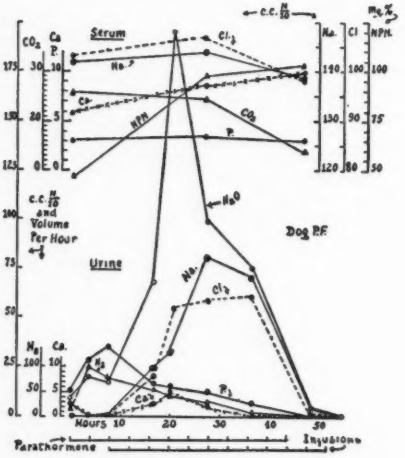


Fig. 4

Fig. 3. Effect of parathyroid extract and of saline injections on the composition of blood and urine of dog *T* (wt. 31 kg.).

Fig. 4. Effect of parathyroid extract and of glucose and saline on the chemical composition of the blood and urine in dog *P.F.* (wt. 18.5 kg.).

parently not altered by the diuresis. The excretion of phosphorus and nitrogen in this animal increased appreciably, but never to the same extent as that of sodium and chloride. In this dog, the phosphorus excretion preceded the excretion of water, sodium, and chloride by several hours, but declined much sooner. In another animal, dog *D,* the excretion of phosphorus in the urine followed the curves for water and chloride excretions, as shown in figure 1. This dog weighed only 13.5 kg. and received 4-hourly injections of 100 U of parathyroid extract, or 7.5 U per kg., which was 2.3 times the dose per kg. received by dog *P.* With this amount of the extract the diuresis began almost immediately (retention catheter), reaching its highest levels at 4 hours and declining just as rapidly, so that exitus occurred in 40 instead of 60 hours, as in dog *P.* In this instance, the phosphate followed the trend of water ex-

cretion, and the excretion of chloride was less than that of phosphate and nitrogen. The reason for this difference is not entirely clear.

The autopsy findings in these 2 animals showed calcification in the kidneys, heart muscle, and the mucosa of the stomach. However, from the sections of the kidneys one would not have thought that 'blockage' of the tubules with lime salts could have been the cause of the anuria.

Two other dogs similarly treated died at 60 and 56 hours respectively. They likewise showed calcification in the kidneys and chemical findings in the blood and urine similar to those found in dog *P*.

In order to ascertain whether or not the calcification in the kidneys is responsible for the anuria resulting from parathyroid extract overdosage, 2 small puppies were injected with 50 U of the extract every 4 hours and allowed to

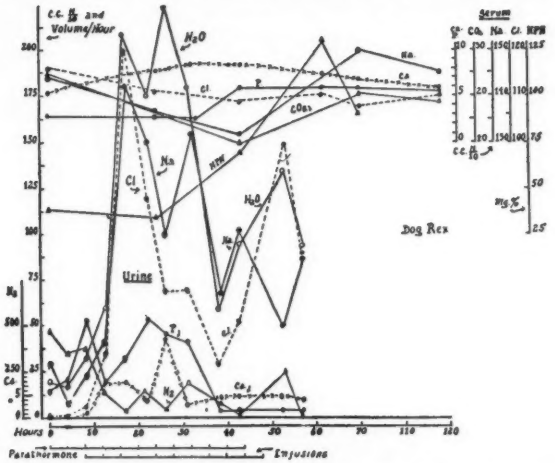


Fig. 5. Effect of parathyroid extract and saline injections on the chemical composition of blood and urine of dog *R*. This animal weighed 30 kg. and received 12 four-hour injections of 100 U of parathyroid extract and 11 infusions of 200 cc. of physiological saline solution, beginning after the third injection of the extract (see text).

drink water. This large dose of about 20 U of the extract per kg. of body weight and the loss of electrolytes by vomiting made the animals quite ill. One died 28 hours, and the other 30 hours, after the initial injections, anuria developing several hours before exitus. The concentrations of calcium and inorganic phosphorus in the serum, shortly before exitus, were: Ca, 19.5 and 18.0; P, 13.1 and 12.4 mg. per cent respectively. The autopsy revealed kidneys engorged but containing no gross or microscopic calcic deposits. Evidently, anuria can occur in parathyroid extract overdosage without calcification being present in the kidney tubules.

*Effect of saline and glucose on the chemical composition of blood and urine and on the symptoms of parathyroid overdosage in dogs.* The demonstration that the anuria and death, which occurs in dogs injected with repeated moderately large doses of parathyroid extract, is associated with a loss of water and electrolytes by diuresis raised the question as to what effect replace-

ment of water and electrolytes would have on the symptom-complex of parathyroid extract overdosage. Accordingly, we tried this therapy and studied the chemical composition of the blood and urine in dogs injected with the extract and treated with saline solutions. In the first dog (*T*), only 6 four-hourly injections of 100 U of parathyroid extract were made, lest the animal become too ill to be benefited by the therapy, and when it was found that the therapy was effectual in this animal, the injections in the second dog (*R*) were increased to 12. Each animal was treated with approximately 200 cc. of physiological saline solution intravenously about every 4 hours, at a rate of about 10 cc. per minute. Dog *T* was treated from the second to the last injections of the extract, i.e., it received 5 injections of saline;[2] and dog *R* was given 11

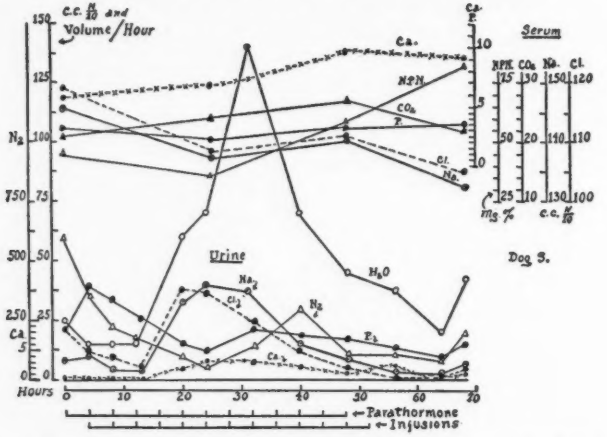


Fig. 6. Effect of parathyroid extract and of glucose injections on the chemical composition of blood and urine of dog *S*. This dog weighed 29 kg. and received 12 four-hourly injections of 100 U of parathyroid hormone and 11 infusions of 200 cc. of 6.0 per cent glucose. Note the lesser loss of sodium and chloride as compared with water, the definite reduction of sodium and chloride, and the increase in the nonprotein nitrogen concentrations in the serum.

infusions, beginning with the third injection of the extract. Both of these animals survived the treatment and appeared outwardly well shortly after the experiments. The results of these studies are shown graphically in figures 3 and 5. Figure 3 which shows the chemical composition of the blood and urine of dog *T*, suggests that, although the parathyroid extract and the saline injections produced a marked water and electrolyte diuresis, they did not lead to anuria or oliguria, as in the untreated animals. In fact, the hourly excretion of urine at the time the experiment was discontinued was still much higher than the rate of urine formation during the control period. The animal appeared well during the entire experimental period, in spite of the fact that the concentration of calcium in the serum reached as high as 15.7 mg. per cent (7.85 cc. N/10). The other constituents of the blood were affected but little. There was a slight reduction in the chloride concentration, in spite of therapy with NaCl, and also a moderate increase in the nonprotein nitrogen concentra-

[2] Tyrode solution from which calcium and phosphorus were omitted.

tion after therapy was discontinued. However, 3 days after the discontinuance of the experiment, the chemical constituents of the blood were within normal limits, except for a slight elevation in nonprotein nitrogen (Ca, 5.2; P, 2.5; Cl, 113; $CO_2$, 23.0 cc. N/10; and nonprotein nitrogen 57 mg. per cent). These chemical changes in the blood may have been due to the marked diuresis, which persisted for many hours even after the discontinuance of therapy.

The chemical findings in the blood and urine of dog *R*, presented in figure 5, demonstrate that in spite of the marked fluctuation in the chemical composition of the blood, and in the excretion of water, sodium, chloride, and phosphorus, the animal was kept in a state of diuresis through the entire experimental period thereby preventing the anuria and its consequences. These

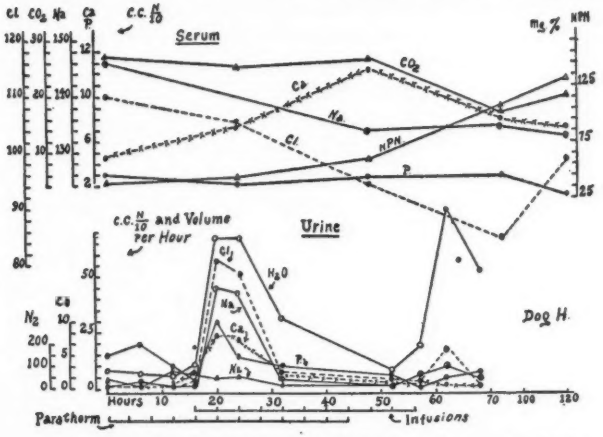


Fig. 7. Effect of parathyroid extract and of glucose injections on the chemical composition of the blood and urine of dog *H*. This dog weighed 18 kg. and received 12 four-hour injections of 50 U of parathyroid extract and 13 infusions of 200 cc. of 6.0 per cent glucose, beginning with the second injection of the extract. This animal appeared more ill than dog *H*, similarly treated. Note the hypochloremia and moderate loss of sodium and chloride in the urine during the height of the diuresis, and their decline as the diuresis ebbed (see text).

fluctuations may have been due to the fact that the animal vomited several times during the experiment, and thereby lost water and electrolytes, and also to the fact that the intravenous therapy was not 'continuous', as was the excretion of urine. But since it is practically impossible to administer a continuous intravenous 'drip' to unanesthetized dogs, the clyses of 200 cc. were administered at fairly rapid rates, within periods of from 15 to 20 minutes. The fluctuations in the acid-base equilibrium, resulting from the therapy and the vomiting are evident from the reciprocal increases and decreases of the principle anions in the serum. These chemical changes persisted even after the discontinuance of treatment, and they may have been due to the prolonged effects of the extract and of the saline in maintaining the diuresis. Considering the number of injections of parathyroid extract and the fluctuations in the blood and urinary constituents, it is remarkable that the animal survived at all. Yet the dog appeared only slightly ill during the experiment and entirely well for months thereafter.

Saline therapy was tried in a third dog, but it had to be discontinued because during the experiment the dog was found to be suffering from distemper. The animal died shortly after the experiment was begun and an autopsy revealed a far-advanced pneumonic process in both lungs.

The beneficial results obtained with saline therapy in the two dogs raised the question as to the specificity of the treatment, and as to what extent other substances able to promote diuresis by mobilizing electrolytes from the tissues could avert anuria and fatal termination in dogs suffering from overdosage with parathyroid extract. Two dogs were therefore treated as outlined above, except that a 6.0 per cent solution of glucose was substituted for the salt solution. The first dog (*S*) received 13 four-hourly injections of 50 U of parathyroid extract and 13 intravenous injections of glucose, starting after the second injection of the extract. The second dog (*H*) received 12 injections of 60 U of the extract and 11 intravenous treatments, starting after the fifth injection of the extract. Both animals retched, vomited small amounts of fluid, and appeared sickly, but they were apparently in good health within a few days after the experiments were discontinued.

The chemical findings in the blood and urine of dog *H* are shown in figure 6, and those of dog *S* in figure 7. Figure 6 shows that, in spite of the definite hypercalcemia and moderate hypochloremia, the urinary flow was not impaired. There was an increase in the excretion of sodium and chloride, but this was moderate in comparison with the larger water loss, so that the concentrations of the electrolytes in the urine were low. As such findings were not foreseen, however, the specific gravities and the concentrations of sugar in the urines were not determined, so that it is impossible to tell whether they were of low or fairly normal specific gravities, due to the excretion of glucose.

However, it is significant that, in spite of the loss of electrolytes, the maintenance of a sufficient urinary flow by the glucose infusions prevented the fatal effects of parathyroid extract overdosage. To be sure, such therapy can not entirely replace the lost electrolytes, as may be judged from the tendency of the sodium and chloride concentrations in the serum to decrease and the nonprotein nitrogen to increase. It is now well established that the infusion of glucose, without treatment with parathyroid extract, produces a loss of electrolytes from the body, for which reason it is not at all surprising that their combined use resulted in a loss of appreciable amounts of these ions in the urine. In dog *S*, the stores of electrolytes in the body fluids were apparently adequate, and hence the glucose therapy was quite efficacious. However, in dog *H* (fig. 7), such therapy rendered the animal quite ill, possibly because its stores of electrolytes were poorer, as is evident from the low excretion of Na and Cl in the control urine and in the urine obtained after 4 injections of the extract. Thus, in spite of the fact that the urinary flow was increased by means of the glucose infusions, there was a considerable loss of electrolytes by way of the kidneys, ultimately resulting in a fairly marked hypochloremia and azotemia. The hypochloremia and azotemia were probably aggravated by the moderate degree of vomiting which began at about the 30th, and continued to about the 46th hour, when the infusions were discontinued.

The degree of electrolyte deficit which parathyroid extract may produce

is remarkable (fig. 1), and although the excretion of electrolytes may be minimized by glucose infusions, the loss is appreciable and may result in death. It is also important to realize that more salt may sometimes be lost than is administered. An example of the latter occurrence is illustrated in figure 4, showing the chemical changes in the blood and urine of dog *P.F.* This dog received 12 four-hourly injections of parathyroid extract, and was treated with 12 intravenous infusions of 100 cc. each of normal saline and of 6 per cent glucose, beginning after the third injection of the extract. It was hoped that, by maintaining an active urinary flow and the substitution of electrolytes for some of the glucose in the infusion, the symptoms of overdosage would be averted. But our expectation did not materialize. Although therapy produced a profuse diuresis, which continued for about 36 hours, the urinary flow gradually subsided to a state of oliguria, the animal dying 55 hours after the initial injection of the extract. On analyzing the chemical data of the blood and urine, the reason for the therapy failing in this animal became evident. We did not realize, at the time the experiment was conducted, that the amounts of water, sodium, and chloride, lost during the diuresis and resulting from the extract injections, were far greater than the amounts replaced by the infusions. Thus, in addition to water lost by respiration, the total water excreted by the kidneys in this dog was 3636 cc., and the infusions amounted to only 2450 cc. Obviously, the loss of water and electrolytes in excess of that administered was so pronounced that, for practical purposes, the therapy was of little or no value at all. As mentioned previously, had we been able to maintain a continuous intravenous injection of water and salts, at least in amounts approximating that lost in the urine, the dog might have survived. In contrast, dog *H*, although receiving 2200 cc. of glucose without saline, excreted only 1664 cc. of urine, but the depletion of about 850 cc. N/10 of chloride by diuresis, not replaced, was sufficient to produce the symptoms of azotemia.

## COMMENT

The results of the chemical analyses of the blood and urine, showing that repeated injections of parathyroid extract produce a loss of water and electrolytes, suggest that the symptoms in parathyroid overdosage are due to extreme dehydration. This suggestion is borne out by the experiments in which the symptoms of overdosage with parathyroid extract were prevented or abated by supplying water and electrolytes during the experiments.

The water and electrolytes lost in the urine were probably derived from the extracellular fluids, since most of the excreted base consisted of sodium. In the one dog (*P*) in which the urinary potassium was determined, its concentration corresponded with that of edema fluid.

Since Erdheim's (14) observations on the relation of parathyroid hyperplasia and rickets, and MacCallum and Voegtlin's (15) finding of decreased concentrations of calcium in the blood after parathyroidectomy, the function and dysfunctions of these glands were generally regarded as being associated with ossification and with calcium and phosphorus metabolism, and but rarely with other physiological functions in the body. The present experiments, and those cited above (8, 11, 12, 13), demonstrate that these glands, or the extract

isolated from them, are concerned not only with lime salt metabolism but also with the distribution of water and electrolytes, other than calcium and phosphorus, in the body. Thus, from figure 2 it may be readily seen that the amount of calcium excreted in the urine, although significantly increased, is but a small fraction of the amounts of sodium and chloride lost at the same time, and hence their importance in relation to the parathyroids is not to be overlooked.

Whether the effect of the extract on water and electrolyte distribution is separate from its influence on lime-salt metabolism, or whether it is an integral part of the process of ossification and decalcification, is difficult to say. As separate fractions of the extract, capable of initiating diuresis and increasing the concentrations of calcium in the blood, have not been isolated it may be assumed that the two functions are probably interrelated. The fact that the salt solutions prevented death and symptoms of overdosage, and did not prevent the hypercalcemia due to the injection of the parathyroid extract would seem to indicate that the essential action of the extract is on the calcium and phosphorus, and that the other effects are secondary.

The chemical changes in the blood and urine in parathyroid extract overdosage resemble those found in adrenal insufficiency (16, 17, 18, 19). In the former, it is the result of hyperfunction of the parathyroids, and in the latter it is due to hypofunction of the adrenal cortex. What effect the injection of cortin would have on parathyroid extract diuresis, or parathyroidectomy on the symptoms of adrenal insufficiency, has not as yet been determined. The diuresis caused by hyperparathyroidism and by adrenal insufficiency differs from the diabetes insipidus caused by hypopituitarism; the urine in the latter disturbance is extremely dilute, containing only small amounts of dissolved solids. What relationship exists between these different endocrine organs in the regulation of water and electrolyte equilibrium in the body remains to be investigated.

The results of therapy with salt solutions indicate that it is possible to abolish all or some of the symptoms of parathyroid overdosage in dogs by replacing the water and electrolytes lost through the kidneys. On theoretical and practical grounds, this therapy may be considered 'specific', but in administering it to human beings or to animals suffering from parathyroid extract overdosage, it is important to gauge the amount of water and electrolytes lost, and to replace it by approximate amounts.

In human beings, the extreme effects of parathyroid overdosage are seldom encountered, because the drug is usually withdrawn as soon as suspicious symptoms, such as nausea, vomiting, hyperpyrexia, slow pulse rate, and cardiac irregularities make their appearance. Lowenburg and Ginsburg (20) encountered a case of parathyroid extract intoxication in a 5-year-old boy, who was given through error 500 instead of 100 I.U. of the extract daily, for a period of 6 days. The concentration of calcium in the serum was 19.4 mg. per cent. Fortunately, the symptoms and the hypercalcemia subsided after the institution of a continuous intravenous 'drip' of saline, a procedure which Lowenburg and Ginsburg thought to be empirical but which, on analysis, seems to be rational and 'specific,' as may be inferred from the results of the present investigation.

The rarity of symptoms of parathyroid extract intoxication in human be-

ings, suffering from hyperfunction of the parathyroids and having constant high concentrations of calcium in the blood, may be explained by the fact that loss of water and electrolytes through the kidneys is constantly restored by the unrestricted intake of food, salt, and water.

Although we were able to prevent death in 2 dogs treated with glucose intravenously, such therapy is not 'specific' and may sometimes accentuate the loss of electrolytes, thereby contributing to the renal failure. As in the dehydration which results from high intestinal obstruction (21) or pancreatic fistulae (22), the use of glucose as a sole therapeutic agent can not be relied upon in parathyroid extract overdosage. In all of these abnormal states of hydration, the aim of therapy is to replace the water and electrolytes lost from the body, preferably by means of a continuous intravenous 'drip' of physiological saline or artificial serum water.

## SUMMARY

The injection of frequent moderately large doses of parathyroid extract into dogs results in a profuse diuresis and a loss of water, sodium, chloride, calcium, and phosphorus from the body. After several hours of diuresis, however, the loss of water and electrolytes gradually diminishes, the loss eventually leading to oliguria, anuria, azotemia, and death. The ill effects of parathyroid overdosage in dogs may be overcome by replacing the water and electrolytes lost through the kidneys.

Although glucose may alleviate the symptoms of parathyroid poisoning in some cases by maintaining a urinary flow, it can not be entirely relied upon to avert fatal end-results, since it may accentuate the mineral loss.

These experiments suggest that dehydration plays an important part in the production of the symptom-complex of parathyroid overdosage.

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# CASE REPORTS

## TREATMENT OF ESSENTIAL HYPERTENSION AND DIABETES MELLITUS BY IRRADIATION OF THE PITUITARY AND ADRENAL REGIONS

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Essential hypertension, diabetes mellitus and obesity constitute a triad which according to Fishberg (1) is often found in the same individual, and not uncommonly one or more of the three are found in several members of the same family. This fact he believes points to a constitutional peculiarity as being responsible for both the diabetes and the hypertension. We believe this constitutional factor is, at least in many cases, a functional abnormality of the pituitary and adrenals. A voluminous mass of evidence from the fields of clinical and experimental medicine, as well as from the laboratories of pathology, supports this idea. Space does not permit the presentation of this evidence here, and it has already been summarized elsewhere (2). Because of the well-known effect of the x-ray on glandular function we believed this agent could be used to correct this functional obnormality with consequent relief of either or both of these syndromes. Whether this idea is correct or not still remains to be determined. A certain number of these cases have apparently been benefited by this type of therapy. It is our experience with these cases, as well as with those unimproved, that we propose to describe now.

This is not the first time the x-ray has been used in the treatment of these two syndromes. Cushing reported the correction of an intractable diabetes in a case of basophilism following irradiation of the pituitary (3). He also reported symptomatic relief and some reduction in blood pressure in another case of basophilism following irradiation of the pituitary (3). The French (4) reported some success by irradiating the adrenals as a treatment for hypertension. Selle, Westra and Johnson (5) reported that irradiation damaged the pituitary and adjacent structures without influencing the carbohydrate tolerance of pancreatectomized dogs. However, their dosage varied from 750 to 2,025 r for a single treatment and consequently can hardly be compared with the work reported here because of the very great difference in the dosages employed. Baird, Lingley and Palmer (6) reported 8 patients with severe essential hypertension treated by roentgen irradiation over the pituitary and adrenals or both without markedly favorable effects on blood pressure or symptoms. They employed doses very much larger than were used in the series we are reporting. However, it is believed that this is the first time irradiation of both of these structures has been proposed for the treatment of these syndromes where only a function disturbance of the pituitary and adrenals was postulated as responsible for them.

Since this work was begun, Barnes (7) took a series of pancreatectomized dogs and, after determining their insulin requirement, irradiated the pituitary and adrenal regions on the same machine that we use in treating our patients. The dogs experienced a reduction in their insulin requirement averaging 50 per cent. Unfortunately, however, human diabetics have not responded so well as the dogs. About one-third of them have shown no improvement of any kind; another third experienced some symptomatic relief but with very slight improvement in their carbohydrate tolerance; another third showed improvement in carbohydrate tolerance and a number were able to discontinue the use of insulin.

For example, Mrs. F., aged 58 had had diabetes for a number of years and had been in the hospital (Illinois Central) for acidosis and infections. She had not been able to work for 2 years. She was taking 20 U of insulin per day when treatment was started. After 4 x-ray treatments she discontinued the use of insulin, and increased her diet slightly—just how much is not known, but she has gained 25 pounds. She returned to work in an office and has continued ever since. Her blood sugar readings have been as follows.

| | | | |
|---|---|---|---|
| 12/26/33 | 160 mg. | 5/1/35 | 135 mg. |
| 1/4/34 | X-ray treatment | 6/14/35 | 160 mg. |
| 1/12/34 | " " | 7/30/35 | 120 mg. |
| 1/19/34 | " " | 9/24/35 | 104 mg. |
| 1/30/34 | " " | 12/?/35 | 180 mg. |
| 4/19/34 | 115 mg. | 1/31/36 | 140 mg. |
| 9/14/34 | X-ray treatment | 4/29/36 | 135 mg. |
| 9/27/34 | 120 mg. | 7/30/36 | 110 mg. |
| 11/30/34 | 140 mg. | 10/30/36 | 140 mg. |
| 12/3/34 | X-ray treatment | 2/13/37 | 165 mg. |
| 1/29/35 | 135 mg. | 3/1/37 | 165 mg. |
| 1/30/35 | X-ray treatment | 3/31/37 | 125 mg. |
| 4/15/35 | 160 mg. | 6/3/37 | 125 mg. |

The difference between this patient and diabetics who showed no response to this treatment is something we have been unable to determine.

In this series there are 334 patients who had hypertension; of this number 32 were also victims of diabetes. The response to this form of therapy of patients having both hypertension and diabetes is shown in table 1.

TABLE 1. HYPERTENSION AND DIABETES

| | |
|---|---|
| Improved as to both conditions | 9 |
| Improved as to hypertension only | 8 |
| Improved as to diabetes only | 3 |
| Insufficient treatment | 6 |
| Unable to follow | 2 |
| Unimproved | 2 |
| Improved but relapsed | 2 |
| | 32 |

The first of these patients was a Pullman conductor, 53 years of age, who came under observation Sept. 6, 1933. His diabetes had been discovered at the age of 45 when he was making a slow recovery from an attack of mumps. His hypertension was discovered 2 years later. He had never taken insulin and had excreted sugar most of the time. In the previous 8 years he had frequently exhibited a fever of 102 or 103 degrees, lasting for several weeks, the cause of which had never been determined. He had been hospitalized a number of times in different institutions so that his status as regards hypertension and diabetes was well established by a

number of independent observers. He complained of headache, diplopia and blind spells. The Eye Department reported retrobulbar neuritis.

His glycosuria and hyperglycemia disappeared after a few x-ray treatments and have recurred but once since. His symptoms were promptly relieved and his blood pressure declined from 225/120 to 150/85. On a number of occasions it has risen to its previous high level associated with the old complaints of headache, diplopia and blind spells. On one of these occasions he was in the New Orleans Hospital, which reported his blood sugar as 80 mg. His blood pressure varied from a low of 140/80 on July 3, 1934, up to its original level. For a good while it has ranged from 170 to 180 systolic and 80 to 90 diastolic. There has been no return of glycosuria or hyperglycemia. He has had only one attack of fever of undetermined origin since treatment was begun.

The last of these patients was a 52-year-old lawyer referred by Dr. Don Smith of Hope, Arkansas, Dec. 21, 1936. He had had hypertension for some years and his diabetes was discovered in May of 1936 when he was experiencing marked acidosis. When he came under observation he was on a diet of C. 90, P. 60, F. 90 with 22 U of insulin per day. His blood sugar after breakfast and insulin was 94.7 mg. His blood pressure was: right 170/90, left 165/90. He was given one x-ray treatment to both sides of the pituitary and the adrenals on Dec. 22, 1936. His blood pressure declined to 130/80. His blood sugar readings were as follows:

| | | |
|---|---|---|
| 12/22/36 | 169.0 mg. | |
| 12/23/36 | 80.6 mg. | |
| 12/24/36 | 74.3 mg. | Insulin stopped |
| 12/26/36 | 151.1 mg. | |
| 12/28/36 | 141.1 mg. | 40 gm. C. added to diet |
| 12/29/36 | 119.6 mg. | |
| | 99. mg. | (Moore Laboratory) |
| 12/30/36 | 87.7 mg. | |
| 12/31/36 | 84.3 mg. | |
| 1/2/37 | 71.1 mg. | |

During the next few months he was reported sugar-free with a blood pressure of about 150. April 26, 1937, his blood sugar after breakfast was 89.6 mg. In the meantime he had gained 20 pounds in weight and claimed to feel a great deal better. His blood pressure was 155/85 in both arms.

In most cases it was not possible to determine which of these syndromes came first. In a few, however, the history was quite definite. Mrs. P. was known to have had diabetes at the age of 56. It was a mild affair and she was able to take an adequate diet without glycosuria or hyperglycemia. Her blood pressure continued normal until she was 64 years of age. It is fairly certain that within a period of 6 months her blood pressure rose from about 130/80 to 220/120 and in approximately the same period her renal threshold for sugar rose to about 300 mg. The blood pressure dropped to around 145/85 after a few x-ray treatments. Her blood sugar, however, responded very slowly and then only temporarily to this treatment. Her renal threshold has become lower since x-ray treatment was started.

Neubauer (8) found hyperglycemia a common accompaniment of arterial hypertension. Herrick (9) was of the opinion that it was present in 10 to 30 per cent of such cases. Fishberg (1) remarks that in patients with essential hypertension the renal threshold for sugar may be very high so that even with well-marked hyperglycemia no sugar appears in the urine. This is a statement

that we have verified in this series of cases. The results of glucose tolerance tests conducted on a number of patients having hypertension only are shown in table 2.

TABLE 2.

| | |
|---|---|
| Blood sugar rose to 170-200 mg. | 37 ( 9 showed glycosuria) |
| Blood sugar rose to over 200 mg. | 60 (19 showed glycosuria) |
| Non-diabetic type of curve | 52 ( 2 showed glycosuria) |
| | 149 (30 showed glycosuria) |

Writers differ in their opinions regarding the relation of hyperthyroidism to hypertension. We have measured the B.M.R. in a series of cases as shown in table 3.

TABLE 3.

| | |
|---|---|
| Lower than —5 per cent | 44 |
| Normal | 51 |
| Over +10 per cent | 49 |
| | 144 |

One of these was a 59-year-old woman who presented the typical picture of hyperthyroidism. Her B.M.R. was +56 per cent. She had undergone a subtotal thyroidectomy in 1928 and another in 1929. A third was being recommended when she came to us in October of 1934. After x-ray treatment directed at her pituitary and adrenals (the thyroid was not irradiated) her blood pressure declined to normal and her B.M.R. to +14 per cent. The symptoms of hyperthyroidism disappeared. However, only a few of these patients having an elevated B.M.R. showed any other signs of hyperthyroidism.

The hypocalcemia and hypercholesteremia reported by some workers (1, 10) has not been observed in this series although these determinations were not made in every case. Very few patients have shown any significant departure from a normal blood count. The phenolsulphonephthalein test has been normal in most cases. In a few cases a higher percentage of the dye was returned after treatment than before. A marked drop in blood pressure was noted in most cases during Stieglitz' amyl nitrite test (10). In most instances an equal drop was noted after treatment. In some cases where the nitrite brought about only a small drop in pressure, treatment was followed by a greater drop. The opposite was also sometimes true.

The response of patients with essential hypertension is shown in table 4.

TABLE 4. HYPERTENSION

| | |
|---|---|
| Improved | 134 |
| Improved but relapsed | 23 |
| Insufficient treatment | 44 |
| Unimproved | 76 |
| Unable to follow | 25 |
| | 302 |

The following may be cited as an example of a case that did not show any favorable response to this treatment:

Dr. Hubert M. English of Gary, Indiana, referred Mrs. Y. to us on Oct. 17, 1934. She was then 34 years old and had known of her hypertension for 10 years. Lately her headaches had grown worse and she had some precordial dis-

tress and shortness of breath. Her blood pressure was: right 260/155, left 260/140. After a few x-ray treatments her blood pressure dropped to 200/120 and has remained near that level. She has had 27 treatments, but there was no further reduction in blood pressure after the first few treatments. There was no symptomatic relief and apparently the progress of her disease was not checked in any way. In the past few months she has had a number of retinal hemorrhages and complains of weakness so that her physical activities are greatly curtailed.

An example of one who responded quite favorably to this treatment is Mrs. D., aged 49, who had known of her hypertension for 3 years. When she was first seen, Feb. 28, 1936, her blood pressure was: right 195/100, left 210/100. She complained of headache and easy fatigability. She had one treatment March 6, 1936. The blood pressure dropped to: right 144/90, left 130/82. March 27, 1936, it had risen to 185/100 in both arms. On this date she was given another treatment. The blood pressure readings since that time are as follows.

| | | |
|---|---|---|
| 4/13/36 | R. 146/86 | L. 150/88 |
| 6/13/36 | 142/78 | 150/78 |
| 7/18/36 | 150/80 | 135/80 |
| 9/4/36 | 150/70 | 155/80 |
| 10/19/36 | 145/90 | 145/88 |
| 1/12/37 | 140/80 | 140/80 |
| 3/5/37 | 165/95 | 165/95 |
| | X-ray treatment | |
| 3/26/37 | 145/85 | 145/85 |
| 5/24/37 | 150/90 | 155/90 |

She is now symptom free.

Because of the wide fluctuations in blood pressure that are known to occur spontaneously in essential hypertension, it is difficult to evaluate any type of therapy. In this series we have had the added difficulty that many patients came from widely scattered points over the entire Illinois Central System and reports of their condition were difficult to obtain after the patients left the hospital. It is for this reason that so many are classified as 'unable to follow.' However, in summarizing our experiences we have attempted to allow for these difficulties and not be led astray by them.

A number of physicians over the United States and Canada have used this method of treatment and allowed us to include their experience in this report. This is shown in table 5.

TABLE 5. EXPERIENCE OF OTHERS

| | Cases | Cases Improved |
|---|---|---|
| Martin | 100 | 80 |
| Baker | 40 | 30 |
| McGuffin | 40 | 30 |
| Finch | 12 | 9 |
| Konantz | 6 | 3 |
| Boswell | 5 | 4 |
| Parkhurst | 5 | 3 |
| Hadley | 4 | 4 |
| Miscellaneous | 56 | 47 |
| | 268 | 210 |

The factors we have found most effective are as follows: 120 kilovolts, 2 mm. aluminum filter, 50 cm. skin target distance, 3 milliamperes, 5 minutes, delivering 50 r units. Each side of the pituitary is treated through a portal 10x10 cm. and the adrenals through a common portal 15x15 cm. All 3 areas are treated on the same day.

The frequency with which treatments are given depends on the blood pressure response. When symptomatic relief and a marked drop in blood pressure occur, no further treatment is given until the blood pressure begins to rise or it becomes evident after a number of observations at approximately weekly intervals that no further fall is to be expected. If no marked fall occurs, treatment is repeated in 1 week. If no satisfactory response occurs after 3 treatments, the time is increased to 7 minutes and about 70 r per area is given.

Most patients say that they experience a pleasant sense of relaxation during or shortly after treatment. Symptoms are usually promptly relieved. The blood pressure sometimes exhibits a considerable drop within 24 or 48 hours after treatment. It occasionally rises for at least 72 hours after treatment. In some cases the systolic exhibits a marked fall while the diastolic is unchanged; in other cases this is just reversed.

Blurring of vision, of which a few patients complained, was promptly relieved. In a few cases retinal lesions have been seen to regress markedly after treatment. Such symptoms as headache, vertigo, tinnitus and pains in the extremities are usually very promptly relieved. This may occur without any significant reduction in blood pressure. Again, if symptomatic relief has occurred with a considerable drop in blood pressure, there may be no return of symptoms even when the blood pressure rises to its original level.

While this method of therapy has been used by a number of physicians in various parts of the United States and Canada, the idea has not generally found favor with the profession. Certain objections have been raised against it. It is generally referred to as 'deep roentgen therapy' to the pituitary and adrenal regions. Stieglitz (11) says: "Recently it has been stated that deep roentgen-ray therapy directed toward the pituitary and adrenal glands causes effective and lasting reduction of the arterial tension, presumably by depressing the secretion of pressor principles. What damage may be done by such radiation to the cranium and back has not yet been ascertained; the studies, like those concerning subtotal adrenalectomy, are still incomplete. General application of the principle of destruction of the endocrine glands is unwarranted. Identification of the etiology in each instance of hypertension is a fundamental prerequisite to curative therapeutics."

As a matter of fact, the dose we use is entirely safe and we have seen no signs of pituitary or adrenal damage, no skin changes, and loss of hair. Doses large enough to be dangerous are followed by unpleasant reactions and are not followed, as a rule, by any reduction in blood pressure.

Roentgenologists have objected to this on the ground that the doses are so small that they could not be effective. Regardless of any theoretical considerations along this line, the fact remains that a large percentage of these people feel better and their blood pressure is lower after the use of these very small doses. This is no new idea in medicine. There are a number of drugs that are useful in small doses but are toxic, harmful or otherwise objectionable in large doses.

One woman to whom much heavier doses had been given died. She had received 1460 r to the pituitary and 777.5 r to the adrenals between September 17 and December 7, 1934 and 288 r to the pituitary and 332 r to the adrenals

between January 2 and January 11, 1936, making a total of 1748 r to the pituitary and 1109.5 r to the adrenals. A complete postmortem examination was made. Neither the pituitary nor the adrenals showed any sign of damage. Adenomas were found in the cortex of both adrenals. Dr. R. W. McNealy removed a section of one adrenal from another woman who received much larger doses of x-ray than we now employ. The pathologist found no sign of x-ray damage in the section removed.

*Precautions.* Treatment should not be administered to a woman within 10 days prior to a menstrual period for even these small doses are apt to be followed by some disturbance of menstrual rhythm and a return of the unpleasant symptoms associated with the hypertension. As already stated, doses should not be given too frequently, only small doses should be used, and treatment should be persistent.

## SUMMARY

This treatment is based on the theory that essential hypertension and diabetes mellitus are due to some functional abnormality of the pituitary or adrenals or both and that the x-ray can be used to correct this functional disturbance with a consequent correction of the hypertension or diabetes or both. In practice, it has been found effective in a very small percentage of diabetics. Improvement has been seen in a much larger percentage of patients having both hypertension and diabetes. In those who were victims of hypertension, symptomatic relief has been secured in more than 75 per cent of the cases. This may occur with or without a marked fall in blood pressure. The hypertension is satisfactorily reduced in about 70 per cent of the cases that are adequately treated and that can be followed until the results of treatment are ascertained. About 20 per cent of these improved cases later relapse. The blood pressure returns to its original level and is so far uninfluenced by this form of therapy. In roughly 25 per cent of the cases there is no response, either symptomatically or in blood pressure readings, to this treatment. In not more than 1 per cent has headache been aggravated. There have been no signs of pituitary, adrenal or other damage.

This work has been made possible by the cooperation of Dr. G. G. Dowdall, Chief Surgeon of the Illinois Central, Dr. LeRoy H. Sloan, Assistant Chief Surgeon in charge of medicine in the Illinois Central Hospital, and other members of the attending and interne staff of that hospital, and particularly by Miss DuBois in the X-Ray Department.

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# TREATMENT OF GENITAL HYPOPLASIA IN THE MALE

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Genital hypoplasia may be limited to the sex organs, but usually it is accompanied by more general manifestations. These not only involve aberrations in physique but may, especially in older patients, lead to feelings of inferiority or inadequacy, neurasthenic behavior, and occasionally to actual reactive depressions. Since attempts to correct the physical defects may merely therefore prove inadequate, a series of cases is presented here which portray the clinical and therapeutic features involved in the treatment of such conditions.

*Case 1. M. S.*, a white male, now 14 years of age, was first seen in June 1933 because of the absence of testes. His family history was non-contributory. He was a full-term child who was delivered by high forceps with a birth weight of 8½ lbs. He was always obese and was troubled with polydypsia, polyuria and enuresis since early childhood.

Physically he was tall and obese with pelvic-shoulder girdle adiposity, marked genu valgum, and the testes bilaterally absent from the scrotum and inguinal canals. The penis was small (1.5 cm.). Roentgenological study of the skull, chest and long bones revealed normal findings. The basal metabolic rate was —16 per cent; all other routine laboratory findings were normal. In personality he was dull, shy, self-sufficient and unsociable, and possessed an intelligence quotient (Binet-Simon) of 81.

The diagnosis was: *a*) Dystrophia adiposo-genitalis and neurohypophyseal dysfunction as evidenced by the infantile genitalia, genu valgum, pelvic-shoulder-girdle adiposity etc. *b*) Anterior lobe dyspituitarism with insufficient basophilic secretion (infantile genitalia) and overaction of the eosinophilic components (height above maximum normal). *c*). Bilateral (intra-abdominal) undescended testes. *d*). Mental retardation. *e*). Dysplastic constitution.

Daily intra-muscular injections of the water soluble fraction of pregnancy urine were given beginning with 50 R.U. and gradually increasing to 250 R.U. daily. Within a week both testes entered the canals. Posterior lobe extract (pituitrin-o, 1 cc. twice weekly) and thyroid (½ gr. t.i.d.) were then added because of the Fröhlich syndrome and low B.M.R. After 5 months of treatment, both testes had entered the scrotum and had become progressively larger. The penis likewise became larger, and the body form approached normal. His previous shyness gave way to aggressiveness, and he soon took to boys' games. The B.M.R. increased to normal, and his intelligence quotient increased to 98. With the first injection of pituitrin, enuresis stopped in this patient.

During the last year, the child has undergone normal puberty changes and shows the presence of masculine crines, deepening of the voice, and enlargement of genitals to within normal limits, and has made an excellent adjustment to the

society of boys of his own age. At present the right testis is still somewhat smaller than his left, and his skeletal measurements show eunachoid proportions, with the typical relatively long extremities as judged by the length of the span and lower extremities (fig. 1).

*Case 2. E. E.*, a white male aged 15 years, was first seen in April 1934, because of frequent headaches (which became especially marked when visiting with his parents), a peculiar sensitivity to clean clothes, and because of "always feeling hot," complaints that had been present for as long as he could remember. His father was rather short and stocky.

The boy was a full-term child, delivered by mid-forceps. His infantile history was non-contributory. He was a good student, being in the second year of high school at 14 years, but kept himself busy all day long with his studies, for which reason, he said, he was unable to develop friends. Night terrors, fear of darkness, bed wetting, nail biting, stammering when only slightly embarrassed, and temper tantrums were among the neurotic traits present.

Examination disclosed a short, white, obese male with typical Fröhlich features. There were numerous stereotyped twitchings of the head, eyes, nose and hands. The penis was 3 cm. in length. The right testis was in the inguinal canal, and the left testis was rather small. All laboratory findings were normal. His I.Q. (Binet-Simon) was 111.

The diagnosis was: *a*). Neurohypophyseal dysfunction with dystrophia-adiposo genitalis and obesity of the Fröhlich type. *b*). Hypopituitarism with inadequate sex hormone secretion as indicated by infantile penis, unilateral right cryptorchism and underdeveloped left testis, and insufficient growth hormone as indicated by a height below the minimum normal. *c*). Tics of the head, eyes, nose and hands. *d*). Excitability and feelings of inadequacy. *e*). Enuresis on a neuro-endocrine basis. *f*). Dysplastic constitution.

The boy was placed on a low caloric diet, and pituitrin-o was administered intramuscularly twice weekly. Psychotherapy in the form of persuasion and re-education was given, and in addition, the father was spoken to about his childish habit of joking and punning in the presence of the patient, for the child's resentment toward this practice led to mortification which in turn brought on the headaches. Although this treatment continued for 5 months and his bodily form and personality approached a more normal state, his genital condition remained unchanged. The water-soluble fraction of PU was then given twice weekly, beginning with 65 R.U. and increasing to 125 R.U. During the course of another month the penis became elongated, the right testis completely descended, and both testes became enlarged. The boy has now undergone the normal puberty changes, and displays all the usual sexual manifestations.

*Case 3. L. T.*, a white male of 16 years requested treatment because of his shortness and obesity. The family history was irrelevant. The birth was normal, but as an infant he was fat. His school record showed that he attended for 10 years, had failed twice in the lower classes, and was in the 9th grade. Although he managed to get along with children of his own age, he tended to bully the younger ones, was obstinate, sluggish in his movements, and childish in his attitude toward girls.

Physical examination showed a short obese male with typical Fröhlich configuration. The heart and lungs were normal, the colon was huge and distended with fecal material, and roentgenologic study indicated a bone age of 11 years. A Binet-Simon test gave him an I.Q. rating of 62. The B.M.R. was —27 per cent. The penis

was infantile in size, the right testis was absent from the scrotum, and the left testis was extremely small.

The diagnosis was: *a*). Hypothyroidism. *b*). Hypopituitarism (anterior lobe deficiency with dwarfism and genital infantalism, and neurohypophyseal and tuber-

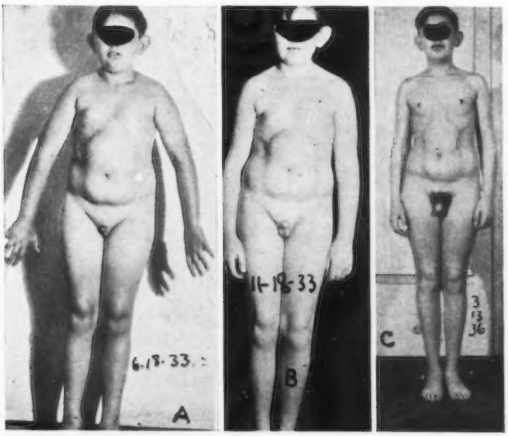
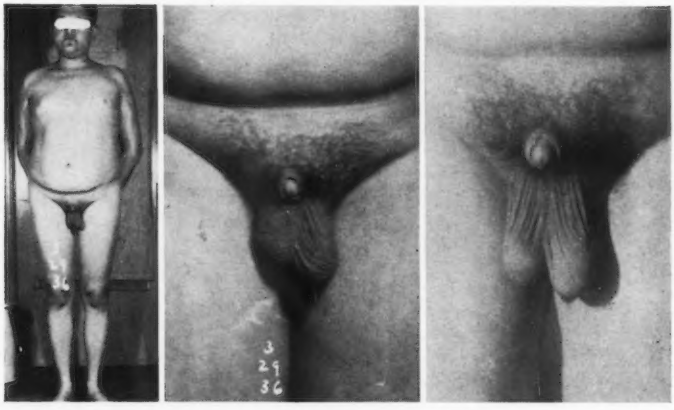



Fig. 1. *A.* Patient (*case 1*) before treatment. *B.* After 5 months treatment. *C.* After puberty.



A B C

Fig. 2. *A.* Patient before treatment, showing dwarfism and eunachoid skeletal disproportion. *B.* The genitals before treatment. *C.* The genitals after 6 months treatment.

cinereum dysfunction with dystrophia adiposo-genitalis). *c*). Mental retardation. *d*). Megalocolon. *e*). Dysplastic constitution.

The patient was given pituitrin-o twice weekly and thyroid, ½ gr. t.i.d. for 3 weeks, and then an anterior pituitary extract rich in the growth hormone was added. These were continued together for 2 months, and although his bodily form changed and growth occurred, his genital condition remained unimproved. Water soluble fraction of PU was then given twice weekly in doses beginning with 65 R.U. and

increasing to 125 R.U. After 4 injections, the right testis became palpable, and after 2 months both testes had become definitely larger. His penis likewise, began to grow.

*Case 4. S. K.*, a white male 12 years, 9 months of age, was first seen in April 1935, because of overweight and small genitalia since birth. A maternal aunt, with underdeveloped genitalia, lives a normal married life, although she has never

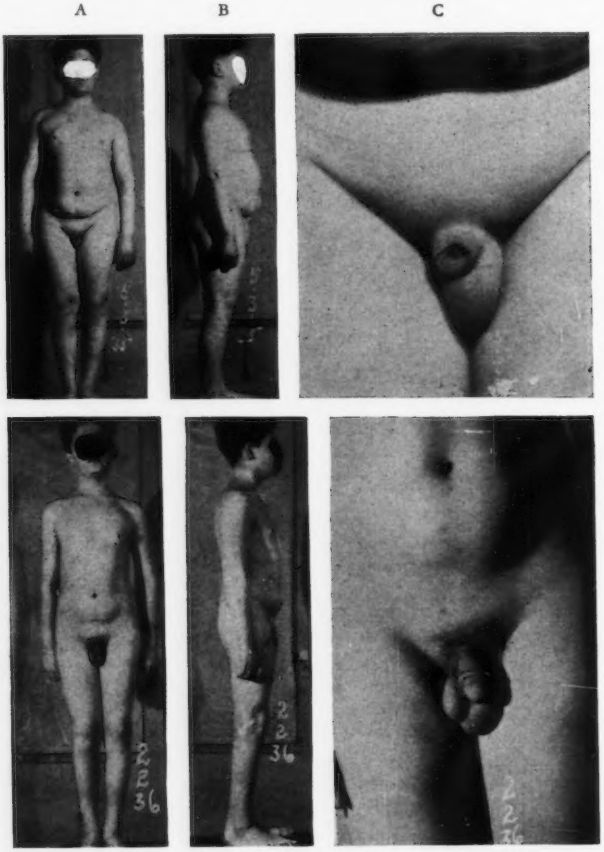



Fig. 3. *A, B, C.* Patient (*case 4*) and genitals, before treatment (May 3, 1935). *D, E, F.* Same patient after 9 months treatment (Feb. 2, 1936).

menstruated or borne any children. The patient was a full-term child, normally delivered, with a birth weight of 9½ lb. He had his first tooth at 11 months, and at the age of one, walked and talked, weighed 30 lb., and was described as having a big head. At 2 years, he had scarlet fever, at 4 years, he had measles and pertussis. At 9 years, mumps set in and he was sent to a hospital, where his mother first noted that the patient's genitals were no larger then those of a patient 1 year of age. He got along poorly with other children, being stubborn and self-sufficient, and although

his school record was excellent (in high school at 12), he was disliked by other children because of his tendency to 'show them up' in the classroom.

On examination, he was found to be a tall boy (61.3 compared to a maximum normal of 60.5 inches), weighing 139 lb., with most of the excess fat over his pelvic-shoulder-girdles, abdomen and mons pubis. His general physical condition was good, but his penis was extremely small (less than 1 cm.), and although his scrotum and testes tended to approach the normal in size these, too, were definitely small (fig. 3).

He was mentally alert, boastful, and realized his intellectual superiority over other children as he did also his genital abnormality, for he refused to undress in the gymnasium, or enter a swimming-pool nude, in the presence of others. On the Binet-Simon test he displayed a mental age of 18 years, 3 months and I.Q. of 143. All laboratory studies led to normal findings.

The diagnosis was: *a*). Anterior pituitary dysfunction with hypereosinophilic and hypobasophilic manifestation as evidenced by growth in excess of maximum normal and infantile genitals respectively. *b*). Dystrophia-adiposo genitalis due to neuro-hypophyseal dysfunction as evidenced by the body configuration; fat distribution. *c*). Mental precociousness in a boy with dysplastic constitution, whose judgment, still in keeping with his age, made him flaunt his mental alertness before the other children. Left to himself, he became more egocentric and self-sufficient.

This patient was placed on a therapeutic régimen consisting of pituitrin-o (1 cc. twice weekly), the water-soluble fraction of PU (125 R.U. twice weekly), and dietary restriction. Psychotherapy, by way of pursuasion and re-education, in order to improve his personality traits and social relations, was also included.

After 9 months of such treatment, his bodily form approached normal (fig. 3), his sex organs showed marked development, he underwent a normal puberty, and made an excellent adjustment toward his work and playfellows. Analysis disclosed his forwardness and conceit in school as an over-compensation,—a result of feeling different and inferior to the other children.

*Case 5. L. W.*, a white male, 12 years of age, was referred for study and treatment because his testes were absent since birth. The maternal grandfather was a diabetic. The patient was a 6½ months' premature baby delivered by breech extraction. At birth he weighed 1.9 lb., and was kept in an incubator for 13 weeks, at which time he weighed 5.3 lb. He was fed by a wet nurse until 6 months of age, and thereafter by bottle. At the age of 10 weeks he developed generalized convulsions to the extent of 91 in 48 hours, which were checked by the administration of bromides. At this time an inguinal hernia was discovered and a truss was prescribed, which he wore until 3 years of age. He had always made excellent progress in school and was athletic in his choice of games.

Physical examination disclosed a tall, slim, figure, with bodily measurements indicative of early eunuchoid gigantism. He had slight bulges over the outer canthi, and his upper jaw markedly overrode the lower. Some of the secondary teeth, which should have been present at his age, were still absent. The penis was small, the crines pubis was present, but of a feminine type. The testes were absent from the scrotum but present in his inguinal canals, the right testis being at the level of the external ring, and the left one in the mid-canal. Neurological examination led to normal findings. Analysis disclosed keen sensitivity to being "different from the other boys," with a tendency to cry while discussing this condition. The intelligence quotient (Binet-Simon) was 112, and a laboratory survey disclosed no significant deviations from the normal.

The diagnosis was: *a*). Anterior pituitary dysfunction as manifested by malocclusion of teeth, infantile penis, and bilaterally undescended testes. *b*). Eunuchoid gigantism. *c*). Dysplastic constitution.

On March 30, 1935, intramuscular injections of the water-soluble fraction of PU were begun, and 24 hours after the first injection of 250 R.U. the right testis descended and appeared to be 1 cm. in diameter. This same dosage was continued daily for 1 week. On the 4th day the left testis had descended to the level of the external ring. On the 8th day 500 R.U., and on the 9th day 625 R.U. were administered. When the patient was seen 2 weeks later, the right testis had completely descended, and the left testis was in the upper part of the scrotum. At this time 875 R.U. were injected in 2 days, and when last seen, 3 weeks later (May 11, 1935), the testes appeared definitely larger.

*Case 6. R. B.*, a white male 9½ years of age, was first seen on April 14, 1936 because of a 'defect in descent of his testes'. Family history was non-contributory.

TABLE 1. SKELETAL MEASUREMENTS IN CASE 6, BEFORE AND AFTER TREATMENT

| | Before Treatment | | After Treatment | |
|---|---|---|---|---|
| | Patient | Normal | Patient | Normal |
| Ht., in. | 56.5 | 50.4–54.0 | 57.3 | 51.4–55.0 |
| Wt., lb. | 77.0 | 54.2–67.8 | 78.0 | 57.0–71.6 |
| Span, in. | 58.0 | 50.3–54.1 | 58.0 | 51.4–55.4 |
| U. M., in. | 28.0 | 25.1–27.5 | 28.5 | 25.5–27.9 |
| L. M., in. | 28.5 | 24.5–26.9 | 28.8 | 25.1–27.5 |

The patient was a normally delivered full-term child weighing 5¾ lb. at birth. The personal history was essentially irrelevant, except that the right testis was seen to recede and come down at irregular intervals.

On physical examination, the child was found to be tall and slim, and skeletal measurements (table 1) disclosed an overgrowth of his long bones. The skin was of normal texture, the hair was coarse, thick and dry. It was significant that while all the incisors present were of the secondary or permanent variety, the upper left lateral incisor was only partially through, and the upper right lateral incisor had not yet erupted.

The penis was of normal length (5 cm.). The scrotum was small and the left testis, which had descended into the scrotum, was about 1 cm. in diameter, whereas the right testis was palpable and high in the inguinal canal. All laboratory findings were normal.

The diagnosis was: *a*). Eunuchoidism with undescended right testis, partially descended left testis, testicular hypoplasia, eunuchoid skeletal proportions and underdeveloped upper lateral incisors. *b*). Probable pituitary sex hormone deficiency. *c*). Dysplastic constitution.

The treatment was limited to the use of an alcoholic-water solution of anterior lobe of the sheep pituitary gland. Dosage began with 1 cc. (100 R.U.) and was quickly increased to 3 cc. 3 times a week. Three weeks later, the testes were both in the scrotum and definitely larger than before treatment. However, one of the most important changes occurred in the child's build. Although he had grown almost an inch taller since treatment had begun, this increase was greater in the trunk than in the long bones. As a matter of fact, the span remained unchanged (table 1).

*Case 7. I. M.*, a white male of 33 years, was first seen in January, 1934, when

he complained of weakness and pain in the chest, and a long-standing fear of jumping from windows. One sister had hypothyroidism and was afraid to sleep in the dark, and his maternal grandfather had diabetes. His immediate complaints seem to have arisen several years ago while he was on a business trip in New York. On this trip when he retired alone in a room in one of the large hotels he awoke about 2.00 A.M., with a feeling of terror lest he jump from the window. Analysis showed that in childhood he had always depended on his mother for his courage and in early youth and adolescence had feared his father, whom he considered brutal. He was always somewhat overweight compared to other children and, until he was married, had had no sexual experience.

He had married six years prior to his first visit and there was one child 6 months of age. He has always had a low sex drive: after several months of marriage coitus occurring about once monthly. No psychogenic factors of recent origin regarding his sexual weakness could be detected.

He was found to be 68¼ inches tall, and weighed 210 lb. There was shoulder-pelvic-girdle distribution of fat. His hips were broad, his breasts pendulous. There was moderate tenderness over the maxillary sinuses, and transillumination revealed clouding. The mons pubis was enlarged, the penis appeared small and somewhat buried in fat, the testes were soft, the prostate gland was very small, and the seminal vesicles were not palpable. Laboratory findings were normal except for his B.M.R., which was —14 per cent.

The diagnosis was: *a*). Hypopituitarism with anterior-lobe sex-hormone deficiency and posterior lobe-hypothalamic dysfunction with obesity. *b*). Anxiety neurosis with obsessions and hypochondriasis in a dysplastic individual. *c*). Maxillary sinusitis.

Treatment consisted of draining the sinuses, and psychotherapy in the form of distributive analysis and synthesis (1); his endocrinopathy was treated by thyroid, pituitrin and, after 6 months, intramuscular injections of the water-soluble fraction of PU (200 R.U.) 3 times weekly. As treatment progressed, the patient gained insight concerning his fears, and yet when last seen in November, 1935, he was not completely cured of these: as one fear was dissolved, another was grasped, but with less intensity than the previous one. His body build swung toward the normal, and his B.M.R. came within normal limits, although sex pace did not increase until the PU extract was used. Then sexual intercourse was successfully practiced two and three times a week, a pace that was maintained as long as treatment with this substance was continued.

*Case 8. P. C.*, a white male 31 years of age, was first seen on March 25, 1936, at which time he complained of small genitalia. His family history was non-contributory. There were no major illnesses in his childhood. He first became aware of his condition when he was 10, at which time a physician told him that development would occur later. He was thin until he was 13, and then a scant crines pubis appeared, and he began to take on fat. Disappointment in attempted treatment led to feelings of inferiority and depression.

He was rather short (62.5 inches) and weighed 164 lb. His lower measurement (symphysis pubis to bottom of feet) was 4½ inches more than his upper measurement (symphysis pubis to top of head). His obesity was of the pelvic-shoulder-girdle variety, some fat also being distributed over the breasts, abdomen and trochanters. His legs showed a lateral curvature, and there was moderate genu valgum and flat feet (fig. 2, *A*).

The skin was very smooth, with no hair on the face, and there were only a few

sparse hairs in the axillae. The voice was high-pitched. None of the third molars had yet erupted, and the upper lateral incisors were very small.

His genitalia were markedly underdeveloped, there being a sparse crines pubis with escutcheon pointing downward. The penis measured 2 cm. in length and 1 cm. in diameter. There was no evidence of hypospadias; the prepuce had been partially removed by circumcision. The testes were present within the scrotum, but only about the size of a pigeon's egg. The epididymi were small, and the prostate and seminal vescicles were not palpable (fig. 2, *B.*). Neurological findings were entirely normal. Roentgenograph studies were non-contributory, and the B.M.R. was —14 per cent.

The diagnosis was: *a*). Hypopituitarism (hypobasophilism with sex hormone deficiency, and hypoeosinophilism with growth defect). *b*). hypothyroidism secondary in character, with lowered B.M.R. and unerupted third molars; *c*). Hypogonadism (probably secondary) with infantile penis, feminine crines, small testes, high-pitched voice, hairless face, eunuchoid skeletal proportions, small lateral incisors, impalpable prostate and seminal vesicles. *d*). Dysplastic constitution with Fröhlich configuration due to neurohypophyseal involvement, as evidenced by genital dystrophy, genu valgum and pes planus.

The treatment included a diet of 90 gm. carbohydrates, 50 gm. protein and 60 gm. fat (1100 cal.). In addition, thyroid (½ gr. t.i.d.), pituitrin-o (1 cc. 3 times weekly and PU exact (300 R.U. 3 times weekly) were given. During the next few months there was a definite recession of fat, and a perceptible increase in penile and testicular size, so that at present the penis is 5 cm. in length, and the prostate palpable, although small. There has been as yet, no evidence of change in hair distribution, although the voice has definitely deepened. The patient is still under active treatment (fig. 2, *C*).

## DISCUSSION

From a consideration of the material presented, one may appreciate that treatment of genital hypoplasia cannot be limited to the use of one drug or hormone, or to any other single procedure. It is only when the patient is studied thoroughly and evaluated as a personality that appropriate measures can be instituted. With infantile patients, thyroid in small doses usually suffices, and the same drug is efficacious in combating the symtoms of hypothyroidism in older patients.

The disturbances in fat and water metabolism were overcome with effective doses of pituitrin-0, administered intramuscularly 2 or 3 times weekly. It is important to begin with small doses (0.2-0.4 cc.) which lead to marked pallor within several minutes because of the constricting effect upon the smooth muscle of the peripheral blood vessel. Since the patient is unaware of this pallor, which may last several hours, it is important to warn him in order to avoid fright or embarrassment. The dosage is then gradually increased until the second effect, abdominal pain, is perceived. This is due to the stimulating action of the pituitrin on the intestinal smooth muscle. Different patients require different doses to produce this intestinal effect. In order to determine the optimal dosage, the amount administered at first is gradually increased, until the patient reports the occurrence of a bowel movement about half an hour, or an hour, after the injection. The dosage is then diminished to just under this amount, which will constitute the optimal dosage for that particular patient, to be maintained at the same level. It is true that the reports of some

able investigators (2) indicate that pituitrin temporarily lessens the cardiac output, but from clinical experience with the dosage used untoward effects have not yet been encountered.

The actual genital hypoplasia is at present best treated with either the water-soluble fraction of pregnancy urine or, in suitable cases, with the pituitary sex hormone. One may begin with small doses such as 50 R.U., and gradually increase until doses as high as 1000 R.U. are used. The hormone probably acts by stimulating the anterior lobe of the pituitary gland (3) to secrete more pituitary sex hormone. This in turn stimulates the interstitial cells of the testes, which elaborate their own hormone, stimulating the genital and secondary sex structures.

However, any stage of the cycle may be initiated with the appropriate hormones. If, as in *case 6,* there is no marked evidence of primary dyspituitarism, it is not necessary to stimulate the pituitary gland. For that reason, the pituitary sex hormone in the form of an aqueous-alcoholic extract of the sheep anterior-pituitary (gynantrin, Searle) was used.

Since a hormone fails to stimulate the cells from which it originates (4), the sex hormone of the pituitary fails to stimulate this particular gland, but begins the process by stimulating the testis. Whereas follutein or antuitrin-S leads to stimulation of the interstitial testicular tissue, gynantrin directly stimulates both the interstitial and germinal tissues (5).

Although it is well to be cautious in the treatment of endocrine dysfunctions, the hazard of delaying proper treatment may be inferred from this report. A certain patient, who was told as a child that he would outgrow his difficulty, was still, at 32 years of age, the possessor of an infantile penis. In another instance, treatment seemingly prevented the continuation of excessive growth of the long bones.

The treatment of poor sex pace by the use of follutein or antuitrin-S (as carried out in *case* 7) should be undertaken only after painstaking study since the majority of these cases have their origin in psychogenic factors, as is well known, and demand appropriate psychotherapy. In other patients, who are normal so far as can be gathered from neuro-endocrine, physical and psychic examinations, a weak sex urge may not be amenable to treatment. In these cases it is probably best to limit treatment to education so that the patient learns to accept his condition as that which is 'normal' for him.

Finally, it is important that the personality be considered as a whole, and that feelings of inferiority, backwardness, shyness, over-compensations and depressions be treated by appropriate psychotherapy.

## SUMMARY

The treatment of genital hypoplasia in the male during the different age periods of the life cycle is discussed, and a series of illustrative case studies reported.

In infancy, small doses of thyroid have usually been found to suffice for the production of testicular descent. In childhood and older states, gonadotropic hormones have proved useful, and these were either the water-soluble

fraction of pregnancy urine, for patients with hypopituitarism, or the anterior-pituitary sex hormone, which is somewhat better for patients whose pituitary functional defect is less striking. The water-soluble fraction of PU increased the sex pace in one endocrinopathic patient.

Early treatment is advisable to forestall skeletal disproportions and persistent hypoplasias, and, since the accompanying psychic difficulties may be distressing, both the physical defects and the psychological maladaptations should receive appropriate therapeutic attention.

The author acknowledges with appreciation the kind coöperation of the E. R. Squibb & Sons Company and G. D. Searle & Company, in furnishing the water soluble fraction of pregnancy urine (follutein) and the pituitary sex hormone (Gynantrin) respectively.

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## EDITORIAL REVIEW ARTICLE

# THE TREATMENT OF DYSFUNCTIONAL MENSTRUAL DISORDERS[1]

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A complete consideration of menstrual disorders, encompassing an enormous section of medical knowledge and theory, is impossible in a single article. Therefore, variations of the menstrual function which may be ascribed to an organic cause, such as the amenorrhea of pituitary adenomas or the metrorrhagia of cervical carcinoma, are eliminated from this presentation. This review deals with the periodic and qualitative alterations of the menstrual cycle which are loosely termed 'of endocrine origin,' and which are manifested in one of two leading symptoms, amenorrhea or excessive bleeding.

Despite the fact that most physicians have an understanding of the present-day conception of normal menstruation, it is essential to review briefly the cardinal principles of menstrual physiology.[2] The proper action of the anterior lobe of the pituitary gland is fundamental to ovarian function. The anterior hypophysis produces 2 gonad-stimulating hormones which cause ovarian follicle maturation and corpus luteum formation, respectively. Each of the 2 phases of the ovarian cycle, instituted by the hypophyseal hormones, controls a portion of the uterine cycle. The periodic, postmenstrual ripening of a group of ovarian primordial follicles is productive of the follicle hormone, estrogen, which effects the proliferative phase in the uterine mucosa. Thus, during the first half of the cycle, the endometrium grows from a thin, gland-poor layer of cells to a tall, richly glandular tissue. At approximately the mid-interval of the cycle, *one* of the ripening ovarian follicles matures, ovulation occurs and the ruptured follicle is transformed into a corpus luteum. The lutein cells continue to produce estrogen but, in addition, also secrete their own characteristic principle, progestin. During the second half of the cycle, progestin causes the estrogen-primed endometrium to become a hyperemic, functional tissue richly endowed with nutritive secretions necessary to the nidation of a fertilized ovum. If conception does not occur, the corpus luteum disintegrates and, shortly thereafter, the endometrium so earnestly constructed for the housing of a fertilized ovum is dismantled by menstruation.

It is to be noted that this brief description of the menstrual mechanism has glibly omitted certain gaps in our present knowledge, such as the stimulus of the primary (pubertal) pituitary cycle, the mooted existence of an ovulation hormone and the nature of the so-called 'bleeding factor.' These are important

[1] A review of current practices in the Department of Gynecology, Mount Sinai Hospital, Philadelphia.
[2] Detailed reviews of these phases of ovarian physiology will be presented later by other workers.—*Editor.*

considerations, but irrelevant in this discussion. However, a knowledge of the gonadotropic (luteinizing) principle found in the urine of pregnant mammals is of practical interest. This pituitary-like hormone, capable of stimulating the ovaries of animals to a degree similar to that obtained with the gonadotropic principles of the anterior hypophysis, is widely employed as a therapeutic agent.

It should be clear that an understanding of dysfunctional menstrual disturbances demands a working knowledge of the 4 major factors vital to menstruation, namely, the anterior hypophysis, the ovaries, the uterus and the (as yet unknown) 'neuro-endocrine' background. The latter includes all elements, such as thyroid secretion, adreno-cortical hormone and the psyche, which bear either direct or indirect influence on the process of menstruation. The normal menstrual cycle and the reproductive function of the female are entirely dependent upon the integrity of each of these factors. The etiology of any non-organic menstrual disorder usually resides in an altered action of one of the above-mentioned organs. However, inasmuch as all of them are indissolubly linked in function, a disturbance in one very soon leads to secondary alterations of the others. The vast majority, about 75 per cent, of such menstrual derangements have their origin in disturbed action of the anterior hypophysis. A smaller number, some 20 per cent, are caused by primary ovarian disability incident to developmental defects or pelvic inflammation. The remainder, about 5 per cent, arise in a lack of uterine responsiveness, altered thyroid function or adreno-cortical disturbances. Thus, the 2 chief etiologic divisions of both dysfunctional meno-metrorrhagia and amenorrhea are hypophyseal and ovarian. For therapeutic purposes, those patients with a hypofunctioning anterior hypophysis and those with non-responsive ovaries must be differentiated. During the course of the diagnostic analysis, some evaluation of the patient's uterine status, psychic state and thyroid function must be made.

The differential diagnosis between patients with primary ovarian failure and those with pituitary hypofunction is generally not difficult. The very appearance of the 2 groups is strikingly different. The primary hypogonad patient is characteristically underweight, hyperemotional, thoroughly feminine in demeanor, and the frequent victim of that sadistic triad, dyspareunia, dysmenorrhea and dyspepsia. The patient with hypopituitarism (excluding the uncommon Lorain-Levi type) shows mammary-mons-girdle obesity, hypertrichosis and a well-balanced nervous system. Certain laboratory procedures, more especially the determination of the blood and urine content of hypophyseal sex hormone, may aid the differentiation. For instance, the finding of the follicle-stimulating or the luteinizing factor in the urine immediately eliminates the presence of pituitary hypofunction.

## THERAPEUTIC PRINCIPLES

*Elimination of organic disease.* The symptomatologies of organic and non-organic menstrual irregularities are essentially similar. The differential diagnosis must thoroughly eliminate all possible organic lesions before any menstrual disorder may be assumed to arise in an alteration of function. One of the

most reliable diagnostic agents, in this regard, is uterine curettage. The latter, for instance, is essential to the proper treatment of uterine bleeding of the menopause and post-menopause. No physician has the moral right to presume that such an abnormality is either benign or malignant without recourse to measures aiding the establishment of a positive diagnosis.

*Evaluation of the endocrinopathy.* A knowledge of the normal menstrual cycle and of the endocrine mechanisms which cause menstrual irregularities is of paramount importance. When confronted with such an abnormality, the various endocrine factors involved must be evaluated in order that the required remedial agents may be directed to the primary site of the disorder. Without an appreciation of this dictum, any therapy proposed for dysfunctional uterine bleeding or amenorrhea remains perfunctory and haphazard.

*Individualization.* Equally important with the elimination of organic disease and the determination of the primary site of dysfunction is the knowledge that no two patients ever present precisely the same problem. Coupled with the principle that menstrual disorders should never be treated in a didactic manner is the all-important fact that a patient is not wholly composed of selected endocrine glands. The importance of individual treatment is further emphasized by the varying treatment of the same disorder at different age levels. Thus, it is irrational to treat dysfunctional bleeding in a woman of 47 years by opotherapy, despite the fact that such treatment may be effective in a woman 10 years younger.

*Early treatment.* Inasmuch as malfunction of one endocrine gland may cause functional disturbances in several others, the principle of early treatment is axiomatic. Far-reaching constitutional effects may follow in the wake of innocuous appearing menstrual disorders. Persistent essential dysmenorrhea often causes an inferiority complex which may be the forerunner of an anxiety neurosis. Menorrhagia or metrorrhagia may lead to serious secondary anemia. Amenorrhea, occasioned by a lack of ovarian function, may culminate in marked uterine atrophy which may in turn so stubbornly resist any subsequent ovarian stimulation as to become itself a cause of continued amenorrhea. If this vicious circle mechanism were more widely appreciated, earlier treatment of the customarily unchallenged adolescent amenorrhea would result in a lessened incidence of adult amenorrhea.

## THERAPEUTIC AGENTS

Space does not permit a complete discussion of the experimental and clinical backgrounds of the many therapeutic agents currently advocated for non-organic menstrual disorders. Although some of the following statements may appear didactic, they are well-supported by experience.

*1. General measures.* It is obvious that all non-specific, hygienic measures which aid the patient to attain physical and mental normality should be freely exhibited. Such measures are especially valuable in the management of the menstrual disorders of young girls afflicted with the usual adolescent bugbears, namely, too arduous school-work, poorly balanced diet and the lack of outdoor exercise. The importance of a proper diet to women of childbearing

age is amply illustrated by the disastrous effects of dietary fads on menstruation, by the phenomenon of 'kriegsamenorrhöe,' and by the studies of Reynolds and Macomber (1). Vitamin A, serving in some subtle fashion to increase the state of nutrition (2), is deemed vital to the underweight woman. An increased intake of protein benefits those women suffering from hypophyseal deficiencies. When either obesity or malnourishment is present appropriate measures should be applied.

*2. Estrogen.* The use of ovarian follicular hormone, theelin or other estrogens is contra-indicated in the treatment of any type of dysfunctional uterine hemorrhage. Since the cause of such bleeding is usually a continued and unantagonized action of this very principle, its administration in such instances would be irrational, ineffective and probably harmful.

The objective of estrogen therapy in amenorrhea, oligomenorrhea or hypomenorrhea is to combat uterine hypoplasia. Certain amenorrheic women, possessing a fair degree of ovarian function, as evidenced by repeated titrations of the blood and urine for estrogens, and having an atrophic uterus as a residuum of an earlier ovarian deficiency, usually respond to estrogen therapy alone. As previously noted, the uterine atrophy is overcome and the menstrual cycle is re-established (3). However, the average amenorrheic woman, failing to show the cyclic presence of blood and urine estrogen and having uterine atrophy, is suffering from ovarian hypofunction, either primary or secondary to pituitary deficiency, and requires specific agents directed to the organ primarily responsible for the disorder. In such patients, an estrogen is useful as an *adjuvant* in order to stimulate uterine growth in anticipation of a regulated hypophyseal-ovarian mechanism.

The dosage of estrogen in amenorrhea should be that amount which will raise and maintain the quantity of circulating estrogen to the normal premenstrual level. When administered for this purpose, estrogen is best given orally in doses of from 1000 to 2000 I.U. daily for 8 to 12 weeks. Ample data have accumulated (3, 4, 5) proving the effectiveness of orally administered estrogens. The chief advantage of the oral route, aside from the economic factor, is that it more closely imitates nature by supplying small quantities of the hormone continuously. When administered hypodermically in amenorrhea, the dose of estrogen should be approximately 5000 I.U.[3] twice weekly. A hypodermic dose greater than 10,000 I.U. twice weekly should be avoided because of the hypophyseal inhibition caused by such doses, and the possibility of defeating the purpose of other agents simultaneously employed to stimulate hypophyseal function. In occasional instances, large hypodermic doses of estrogen administered during a very short period may be indicated (3).

*3. Anterior pituitary and anterior pituitary-like preparations.* Extracts of the anterior hypophysis containing both gonadotropic hormones, the follicle-stimulating and the luteinizing factors, and preparations of pregnancy urine (prolan) containing only the latter have a wide field of application in dysfunctional menstrual disorders. The administration of the luteinizing factor (prolan or pituitary-like principle) is indicated in the dysfunctional uterine bleeding of puberty and childbearing age. The dysfunctional uterine bleeding

[3] In oil.

of the menopause, being associated with sclerotic ovarian changes and independent of hypophyseal deficiency, should not be treated by this substance. The clinical experience of a host of observers (6, 7, 8, 9, 10, 11) warrants the continued use of the luteinizing factor in dysfunctional uterine bleeding of women under 40 years of age, despite the present lack of unanimity concerning the effects of various doses on the ovaries and endometrium (12, 13, 14, 15). From 65 to 80 per cent of such patients are restored to temporary menstrual normality by intramuscular injections of 200 R.U. of the luteinizing principle, given daily during the bleeding phase and semi-weekly for 6 to 8 weeks after the bleeding has ceased. However, in more than 50 per cent (9, 10, 16) of those benefited, the bleeding recurs within 6 months because the primary disorder remains unaffected by this treatment. To avoid such recurrences, the concomitant administration of low-dosage irradiation to the pituitary gland and ovaries, or the regulation of an altered metabolism is advisable.

Irregular menstrual periodicity, often associated with bouts of uterine bleeding, and amenorrhea are more adequately treated by the simultaneous administration of both the anterior pituitary and the anterior pituitary-like hormones. The combination of anterior hypophyseal and PU extracts gives a much greater ovarian response in animals (17, 18), and is more effective in the treatment of certain types of amenorrhea (19) than either of the two substances employed individually. Not wholly understood, this phenomenon of augmentation is currently known as the 'synergistic effect' (Evans). In the combined administration of these principles, 200 R.U. of the luteinizing factor (PU extract) and 2 cc. of whole anterior pituitary extract are administered intramuscularly 3 times weekly for 6 to 8 weeks. Such combined substitutive therapy is effective in 50 per cent of the patients with amenorrhea caused by pituitary deficiency (19), but of no avail in the amenorrhea of primary ovarian failure wherein an excess of pituitary sex hormone is already present.

4. *Thyroid extract.* Desiccated thyroid extract is a most effective weapon in the treatment of dysfunctional menstrual disorders. Acting as a specific agent, thyroid extract promptly alleviates the menorrhagia of hypothyroidism which is often the cause of hemorrhages in adolescent girls. In such instances, the B.M.R. may be only moderately lowered to a level between –10 and 20 per cent. However, it has been shown (20, 21, 22) that such minor degrees of hypothyroidism should not be minimized, particularly in the presence of menorrhagia. Thyroid extract is also of value, when employed empirically as a non-specific adjuvant in therapy, because of its augmentation of all cellular activity. It is this 'cellular catalytic action' (Kendall) which engages one to employ desiccated thyroid extract in the treatment of menstrual disorders, despite the presence of a normal B.M.R. The toxicity of overdosage may be avoided by observing 2 principles of thyroid therapy: *a)* the initial dose of desiccated thyroid extract should be small (¼ grain, t.i.d.) irrespective of the B.M.R. The amount administered should be increased rapidly to the point of tolerance, and the maintenance dose continued at a slightly lower level. *b)* the commercial varieties of desiccated thyroid contain different amounts of thyroxin. Thus, to insure accurate dosage for any given patient, the same brand of extract should always be specifically requested in renewal prescriptions.

5. *Low-dosage irradiation.* Low-dosage irradiation of the pituitary gland

and ovaries is one of the most valuable remedial agents in the treatment of both dysfunctional uterine bleeding and amenorrhea. Although it is a purely empiric measure, the safety and value of properly administered roentgenotherapy have been established by the observations of Rubin (23), Drips (24), and Mazer and Spitz (25). The effectiveness of roentgenotherapy in both dysfunctional amenorrhea and meno-metrorrhagia is illustrated in the large series reported by Mazer and Spitz (25). These authors, employing no treatment other than the low-dosage irradiation, noted a favorable response in 50 per cent of the patients with amenorrhea and in 80 per cent of those with meno-metrorrhagia. A word of warning is necessary concerning the roentgen dosage, inasmuch as ovarian overdosage is possible and may do harm. The proper technic, as outlined by Edeiken (26), includes 3 treatments at weekly intervals to both the pelvis (ovaries) and pituitary fossa. "In the patient of average size, the technical factors are 127 kv., 5 ma., 14-inch distance, 5 mm. aluminum filter for 3 to 5 minutes. This is equivalent to 7½ to 12½ per cent S.E.D. or 50 to 80 r" (Edeiken). If no result obtains following the irradiation, a second course of treatment may be given with safety 3 months later. The results of such treatment in amenorrheic patients with uterine atrophy may be enhanced by simultaneously administering estrogen.

6. *Radium.* Intra-uterine radium irradiation is of definite value in the treatment of dysfunctional uterine bleeding of the menopause. While the dysfunctional hemorrhages of young women were formerly treated with moderate doses of intra-uterine radium, current gynecologic practice frowns upon such treatment in women under the age of 40 years. The ovaries of such women are inordinately susceptible to radium. Keene and Payne (27) commented on the high incidence of temporary and permanent amenorrhea following the intra-uterine application of as little as 200 to 500 milligram-hours of radium in women of childbearing age. For this reason, intra-uterine irradiation should not be employed, except as a last resort, in dysfunctional bleeding of the third and fourth decades. However, such bleeding encountered beyond the fourth decade, when cancer is prevalent and when the cessation of ovarian function is not a calamity, should be treated preferably with exploratory uterine curettage and radium irradiation. During the menopausal age, it is imperative to employ the dual procedure, which is both diagnostic and curative. The amount of intra-uterine irradiation, instituted immediately following the exploratory curettage, should approximate 1200 milligram-hours, a dose which will control almost all instances of dysfunctional hemorrhage. If the endometrium recovered is subsequently proved to be malignant, total hysterectomy is the procedure of choice.

The highly satisfactory results of radium treatment in menopausal hemorrhages of benign origin are illustrated in the recent statistics of Anselmino (28), Holden (29), and Norris and Behney (30). Employing from 500 to 1500 milligram-hours of irradiation, the latter authors obtained cures in 83 per cent of 750 women with dysfunctional uterine bleeding. The best results seem to follow the use of 1200 milligram-hours.

7. *Venom.* Recently, the use of mocassin snake venom (Ancistrodon piscivorus) has attained a certain therapeutic vogue in various hemorrhagic condi-

tions (31, 32). The purported rationale of venom in such instances rests on its ability to make animals refractory to the Schwartzman phenomenon (33). Peck and Goldberger (34) and Watkins (35) obtained satisfactory clinical results in patients with dysfunctional uterine bleeding treated with mocassin venom. The venom of individual mocassin snakes varies in activity and deteriorates with time. Therefore, it is best to employ dated, pooled venom obtained from a reliable source. The venom is employed in a 1:3000 dilution and given subcutaneously. The initial dose should be 0.5 cc. and subsequent injections increased rapidly to 1.0 cc. It should be given daily during episodes of bleeding and, when the hemorrhage has been controlled, 2 or 3 times weekly for 3 to 4 months. No constitutional disturbances have been noted with such doses. The severe local reactions manifest by certain individuals who are hypersensitive to the venom may be avoided by a desensitization technic (34).

In employing mocassin venom in selected instances of dysfunctional uterine bleeding in the gynecologic out-patient department of the Mount Sinai Hospital, my colleagues and I were recently impressed with the favorable results obtained in 6 of 11 patients to whom it was administered. Although venom therapy may attain generalized usage in dysfunctional uterine bleeding following further clinical trial, it does not yet merit a definite evaluation.

*8. Progestin.* Shortly after a potent extract of corpus luteum (progestin) became available it was shown to be effective in controlling uterine bleeding of dysfunctional character (36, 37). Although progestin therapy is rational because it counterbalances the estrogen dominance which exists in dysfunctional bleeding, it is only a substitutive treatment and yields a temporary effect. However, as indicated by Mazer and Katz (38) and by Wilson and Elden (39), it may be advisable to use progestin for the temporary arrest of the bleeding in conjunction with other agents employed to correct the basic ovarian or pituitary disturbance. The dosage of the crude corpus luteum extract (progestin) is still purely empiric. Rapid cessation of dysfunctional bleeding has been reported following the administration of 0.04 to 10 I.U. daily (36, 37, 38, 39).

*9. Insulin.* The administration of insulin has been advocated in the treatment of dysfunctional menstrual disorders for more than a decade (40, 41, 42, 43). Insulin seems to be most effective in the menstrual disorders associated with asthenia and malnutrition (cachexia hypophyseopriva and primary hypogonadism). Whether the insulin acts directly by stimulating the ovaries (38), or indirectly by increasing appetite, weight and well-being (44), is not clear. In my experience the restoration of normal menstruation and the improved state of nutrition occur concomitantly with insulin treatment. Despite our limited knowledge of its mode of action, the beneficial effects of insulin in the menstrual disorders of thin, undernourished women warrants its further use in selected cases. The insulin dosage must be individualized according to the patient's tolerance, and proper dietary instructions (high carbohydrate) must be given. The customary dose of insulin recommended for non-diabetics is from 5 to 10 U. before breakfast and supper (40, 41, 42, 43, 44), although Klaften (45) has recently employed much higher amounts.

*10. Surgery.* While the diagnostic value of uterine curettage is unques-

tioned in menstrual disorders, its use as a therapeutic measure is not often justifiable. Temporary relief from hemmorhage will be obtained following curettage because the bleeding surface is removed and, in certain instances of protracted bleeding, such therapy may be desirable. Although uterine curettage is important in any type of climacteric bleeding and desirable in menstrual disorders of women of childbearing age, it cannot be lightly undertaken, however valuable, in young girls. Nevertheless, it should be borne in mind under desperate circumstances.

Halban (46) and Cary (47) have stressed the value of mechanical dilatation of the cervix in the treatment of gonadal deficiencies. It may be that increased ovarian activity follows cervical stimulation or dilatation in humans, as well as in animals (48), and that this may be a factor of value in performing uterine curettage.

Resection of the ovaries, more especially when they are palpably polycystic, is occasionally advocated as a treatment for dysfunctional menstrual disorders. Recently, Robinson (49) and Stein and Leventhal (50) reported excellent results in amenorrhea and meno-metrorrhagia following wedge-shaped ovarian resection. Although such procedure may not be in the same experimental category as endometrial grafting (51), it is not a simple remedy applicable to many women. Any treatment requiring a laparotomy should be relegated to that small group of women whose menstrual disturbances fail to respond to the single or combined use of other remedial agents.

## SUMMARY

The mechanism of normal menstruation is entirely dependent upon the integrity of the anterior hypophysis, ovaries, uterus and 'neuro-endocrine' system. Any disturbance of one or more of these 4 vital factors may result in amenorrhea or meno-metrorrhagia.

The treatment of dysfunctional menstrual disorders should be directed toward the primary site, usually the anterior hypophysis or the ovaries, of the derangement. Several therapeutic measures, each for a different purpose, may be required to restitute normal menstruation.

Certain basic principles of organotherapy must be known before any of the currently employed therapeutic agents may be applied in an aberration of the menstrual function.

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# ASSOCIATION NOTICE

## PRELIMINARY ANNOUNCEMENT OF THE ANNUAL MEETING

The twenty-second annual meeting of The Association for the Study of Internal Secretions will be held in San Francisco, California, on June 13th and 14th, 1938. The St. Francis Hotel will be the headquarters for registration and for the scientific and business sessions.

### PRESENTATION OF PAPERS

Members of the Association and other investigators who wish to present papers will please note the following regulations for the presentation of papers at the coming meetings, especially since they differ in some respects from those of former years.

1. The title of the paper, and a comprehensive abstract *in triplicate,* must reach the President, Dr. F. C. Koch, 947 East 58th St., Chicago, Illinois, not later than March 10, 1938. Regardless of merit, a paper cannot be given a place on the program if the title and abstract have not been submitted before March 10th.

2. Non-members who present papers will appear on the program "by invitation." Non-members are therefore requested to have their titles and abstracts introduced by a member.

3. The titles and abstracts of papers submitted for presentation at the meeting will be considered by the program committee and the final program will be announced about April 1, 1938.

4. Papers will be limited to ten minutes for presentation and five minutes for discussion.

5. All papers presented before the Association or read by title are to be submitted, in completed manuscript form, to ENDOCRINOLOGY, either before or immediately after the meeting. It is to be understood, however, that acceptance of a paper for the program does not obligate the Journal to its acceptance for publication.

You are invited to bring this announcement to the attention of your colleagues and associates who have endocrine researches in progress.

# CURRENT ENDOCRINE LITERATURE

## COLLABORATORS

Fuller Albright
Wayne J. Atwell
Geoffrey Bourne
Israel Bram
John C. Burch
John C. Donaldson
Maurice H. Friedman
Murray B. Gordon
Arthur Grollman
Frank A. Hartman
E. C. Hamblen
F. C. Koch
Edward Larson
J. T. Lewis
Joseph M. Looney
Warren O. Nelson
J. P. Pratt
A. T. Rasmussen
Elmer L. Sevringhaus
L. B. Shpiner
D. L. Thomson
R. L. Zwemer

## ADVANCE ABSTRACTS

**Syndrome characterized by osteitis fibrosa disseminata, areas of pigmentation, and a gonadal dysfunction.** Albright, F., B. Scoville and H. W. Sulkowitch. To appear in this Journal.

Two more case histories in males are recorded of a syndrome characterized by osteitis fibrosa disseminata, areas of cutaneous pigmentation, and marked sexual and somatic precocity when the disease occurs in females. In the case of one male reported, the bone age was precocious, suggesting that even in the male sex some precocity may occur. In another case, on the same side as the cutaneous lesions and the majority of the bone lesions, abdominal and cremasteric reflexes were absent, and there was decreased pain sensation. These findings strengthen the hypothesis that the whole syndrome is due to a disseminated neurological lesion.

**Dynamic tests in thyrotoxicosis.** Bartlett, Jr., W. To appear in this Journal.

Tests in thyrotoxicosis were devised in which the patient performs work of various sorts. The length of time the breath can be held after maximal inspiration and maximal expiration is determined and expressed as an arithmetical index. In normal individuals, the value of this index is about 2-0; in thyrotoxicosis the value of the index decreases to about 1.0. The responses of pulse pressure, pulse rate, and $CO_2$ combining power of the plasma to brief measured exertion constitute further dynamic tests. There are useful indicators as to the necessity for performing thyroidectomy, in the differentiation of thyrotoxicosis in remission from neurosis in the presence of a non-toxic goiter, and in detecting the earliest signs of toxicity in patients receiving thyroid substance for hypothyroidism.

**A study of natural growth and ossification in hereditary dwarf mice.** Boettiger, E., and C. M. Osborn. To appear in this Journal.

The natural growth of hereditary dwarf mice kept under carefully controlled conditions of diet, temperature, and humidity was studied by measuring body weight, body length, and tail length. Growth (average measurements on 375 dwarf mice) is rapid until weaning, although somewhat below that of normal litter mates. Dwarf mice from 17 to 35 days old experience a critical period during which they lose and then regain 16% of their weight. The percentage growth increase in dwarf mice from 25-140 days of age is comparable to that of normal animals during the same period. As in the normal, dwarf growth reaches a plateau at about 140 days. The accumulation of large deposits of fat by older dwarfs (200-500 days) is very pronounced. Full-grown dwarfs are lighter per unit body length than normals. The progressive ossification of the appendicular skeleton of dwarf mice was also investigated by studying the presence and size of 20 ossification centers. The degree of epiphyseal development in dwarf mice 70-80 days of age is similar to that of normal litter mates 25-30 days old. In dwarf mice, extreme variability is characteristic of both growth and ossification.

**Serum proteins before and after operation for hyperthyroidism.** Brown, R. B., and P. M. Mecray. To appear in this Journal.

Serum protein determinations were made both before and after thyroidectomy in a group of 24 hyperthyroid patients. In 14 of the group, the concentration of the serum pro-

teins increased after operation, in excess of the error of the method; in 15 of the 24 cases the pre-operative level was below the accepted normal concentration. An attempt was made to correlate the serum protein concentration with age, sex, B.M.R., weight loss, duration of symptoms, and weight gain following operation. Although suggestive trends were found, the findings were of questionable significance.

**Metabolic, cardiovascular, and biochemical changes associated with experimentally induced hyperthyroidism in schizophrenia.** Cohen, L. H., and J. H. Fierman. To appear in this Journal.

The physiological changes in 8 male schizophrenics, subjected to prolonged periods of large doses of Armour's thyroid U.S.P. ingested orally, are reported. The changes observed were compared with the observations on the same variables made before and after the medication periods, which consisted of slow and rapid thyroidization respectively to a maximum of 15 or 18 gr. per day. In one patient, medication was raised and maintained at a daily dosage of 36 gr. In the patients as a group there was found an increase in the B.M.R., the systolic blood pressure, the pulse pressure, pulse rate, erythrocyte count, total nitrogen of the blood and urine and blood creatine, and a decrease in the diastolic blood pressure and circulation time. Only random variation was observed in oral and rectal temperature, blood sugar level, blood cholesterol level (no deviations from seasonal variation) and creatinine level. Physical symptoms such as excessive perspiration, gastro-intestinal disturbance, tremor, pupillary changes and exophthalmos were consistently lacking.

**Inhibition of hypophyseal gonadotropic activity by sex hormones in parabiotic rats.** Cutuly, E., and E. C. Cutuly. To appear in this Journal.

Hypophyseal gonadotropic hormone from castrated male or female parabiotic rats has been shown to cause *a*), parallel stimulation of germinal and interstitial elements in the testes of hypophysectomized male twins and *b*), continuous vaginal estrus in hypophysectomized female twins. Testosterone propionate or estrone injected into castrated parabionts was found to inhibit the gonadokinetic activity of their pituitaries. The testes of hypophysectomized males joined with such inhibited partners underwent parallel degeneration of both tubular and interstitial tissue, while a hypophysectomized female partner fell into periods of vaginal diestrus. In the hypophysectomized male rats tubular activity was judged in the living animal by testicular size, and interstitial cell activity by scrotal turgescence and flushing. The results can be interpreted in terms of one or two hypophyseal gonadotropic hormones.

**Hyperglycemia without glycosuria.** Davidson, C. F. To appear in this Journal.

Evidence is submitted which indicates that blood sugar concentration, greater than that considered normal, without sugar appearing in the urine, is a frequent occurrence. The presentation recites in detail the methods used in determining blood sugar concentrations. The article reports in tabular form 68 such cases studied during the past 2 years, recording clinic or hospital case number, sex, age, height and weight, amount of glucose given for the test and the blood sugar concentration levels at fasting 30 min., 1 hr., 2 hr., 3 hr., and in some cases 4 hr. after the ingestion of glucose. Clinical improvement, coincident with therapy directed at this metabolic deviation, has often been satisfactory.

**Antihormone studies in boys treated with anterior pituitary-like hormone for genital underdevelopment.** Dorff, G. B. To appear in this Journal.

The sera of 19 boys who received chronic treatment with A.P.L. hormone of the urine of pregnant women for genital underdevelopment were studied for antihormone content. These sera were submitted to Dr. J. B. Collip for study. Twenty samples, taken in all, were found to contain no antihormone. The possible clinical significance is discussed.

**The Cushing syndrome.** Charles W. Dunn. To appear in this Journal.

The paper is a report of the therapeutic results obtained in 11 cases (female) of basophilism treated with progynon-B and proluton. Excellent clinical results were obtained when the combined ovarian hormone therapy was intensively maintained for one year or more. Clinical improvement was manifested by reduction of the hypertension, obesity (without diet regulation), partial loss of the hypertrichosis, relief of physical asthenia and incapacity, head pains and hypo-gonad syndrome. A review of the pathology and recent new findings is included.

**The work performance of untreated hypophysectomized rats.** Ingle, D. J. To appear in this Journal.

When the gastrocnemius muscle of a rat is stimulated immediately following hypophysectomy, the rate of work is normal during the first few hours, but then rapidly decreases to a point at which muscular responsiveness is completely lost. As the interval of time between the operation and the beginning of the work test is increased, the capacity of the muscle to sustain work is decreased. The presence of the posterior lobe of the pituitary body does not protect the animal against the deficiencies in capacity to work, which develop when the anterior lobe is completely removed. If half of the anterior lobe is left intact, the work performance of the rat is carried out at a nearly normal level.

**Electrical excitability (chronaxie) of the rat's uterus, throughout the estrus cycle.** Katzenstein, F. C. To appear in this Journal.

The uterus was excised from adult female rats at various known stages in the estrus cycle. It was suspended from silver chloride electrodes in Tyrode's solution at 35° C., and its electrical excitability determined in terms of chronaxie. Two types of spontaneous uterine contraction, namely 'maximal' and 'submaximal,' were observed, and both could be elicited by electrical stimulation. The methods employed permitted each type to be studied separately. The 'maximal' contractions exhibited a constant and relatively high excitability throughout the estrus cycle. The 'submaximal' contractions, however, showed a cyclic change in excitability corresponding to the estrus cycle. The excitability was highest during estrus and metestrus and lowest during di-estrus and pro-estrus.

**Experimental induction of estrus in the dog.** Leathem, J. H. To appear in this Journal.

All external manifestations of estrus, namely, vulva swelling, bleeding and mating, as well as endometrial hypertrophy, may be induced in the normal dog with either an extract of menopause urine or progynon-B. Induction of estrus with menopause urine extract was successful with mature dogs in anestrus and metestrus, as well as with immature dogs in 14 of the 16 cases tested. This extract produced pure follicle stimulation comparable to the FSH of the anterior pituitary, while progynon-B exerted no obvious effect on the ovary. A cycle accentuated to pro-estrus with menopause urine extract will continue through estrus quite normally, and is followed by a pseudopregnant period, thus duplicating the spontaneous cycle. A pseudopregnant period does not follow estrus induced with progynon-B. Ovulation was not observed with menopause urine extract alone, but injection of follutein on the first day of estrus resulted in pregnancy in 1 of 3 cases.

**Feminization in a male adult with carcinoma of the adrenal cortex.** Levy Simpson, S. To appear in this Journal.

A case of feminization of a male, aged 34, is described together with the partial remission of symptoms following removal of an adrenal carcinoma. Death subsequently followed the recurrence of growth and metastases. The patient excreted a considerable excess of estrogenic hormone (probably estrone), which disappeard after operation and returned with the recurrence of the growth. The histological picture showed certain differences from that of an adrenal carcinoma removed from a virile woman, who secreted an excess of comb-growth hormone. The histological differences may explain why, in the one case, estrogenic hormone and, in the other case, comb-growth hormone were excreted. Alternative explanations are considered.

**Influence of adrenalectomy upon the sex difference in ketosis.** Eton M. MacKay and Richard H. Barnes. To appear in this Journal.

In albino rats the ketonuria of fasting, after a fatty liver has been produced by the diet, due to the administration of sodium acetoacetate or $\beta$-hydroxybutyrate, or higher fatty acids, is always greater in the female sex. Following adrenalectomy this sex difference tends to disappear. The probable explanation of this action is that the sex difference in ketosis is dependent on anterior pituitary activity, and the ketogenic factor of this gland does not act in the absence of the adrenal cortex.

**The sex hormone excretion of children.** Oesting, R. B., and B. Webster. To appear in this Journal.

Complete data are given on a colorimetric assay for male hormones in urine. The androgen and estrogen excretion of 43 boys and 16 girls has been studied. There is a gradual rise in androgen excretion from early childhood to adult life. At puberty there is a distinct rise in

estrogen excretion. We did not find this to be the case for androgens. It is suggested that estrogens play a more significant rôle in epiphyseal closure than androgens.

**Experimentally induced ovulation in dwarf mice.** Clinton M. Osborn. To appear in this Journal.

Female dwarf mice are sterile with sexually underdeveloped reproductive tracts. The ovaries, however, show far more follicular differentiation than is typical of hypophysectomized animals. Ovulation does not occur normally, and consequently corpora lutea are never developed. Experimental ovulation averaging four ova per animal was produced in 9 of 12 dwarf mice by growing the follicles with FSH (subcutaneous injections) followed by 2% to 4% of 1 R.U. of LH (injected intraperitoneally) at the time of the last FSH injection. Possible explanations for the failure to obtain ovulation of large numbers of eggs are suggested.

**A study of the ratio of amount of thelin producing uterine and vaginal estrus.** Szarka, A. J., and G. Kurtz. To appear in this Journal.

To produce the characteristic uterine reaction of estrus in rats 4.8 γ ketohydroxyestrin is required. Thus, in our colony and with the method described in this paper, the uterine unit is 5 to 6 times the vaginal unit. The maximum positivity of the uterine estrus reaction (the complete distention of the uterine horns with fluid) is reached 44 to 50 hours following the first injection, almost 24 hours sooner than the appearance on the vaginal smear of entirely non-nucleated cornified cells characteristic of the vaginal estrus. In the 72nd hour, when the vaginal reaction is at its maximum, the uterine reaction has completely ceased. The biological effect of 4.8 γ crystalline ketohydroxyestrin seems to be equal to the effect of that quantity of hormone produced in the rat during the normal estrus cycle.

**Pituitary dwarfism: treatment with growth hormone.** Taylor, N. M. To appear in this Journal.

The author presents 8 cases of pituitary dwarfism. The treatment employed consisted of antuitrin-G (usually 2 cc. 3 times weekly) together with thyroid extract (in all but 1 case) to the point of individual tolerance. The average period of treatment was 18.6 months, and the average gain in height over this period exceeded the average normal increment by 2.15 inches. The author believes that these responses are sufficiently satisfactory to warrant the assumption that the relatively small doses of growth hormone employed in the 7 favorable cases are adequate to accelerate retarded growth. In none of the cases treated for more than 1 year were phenomena seen which might be interpreted as representing a production of antihormone. Seven of the 8 cases presented serious behavior problems. An exhaustive study of these difficulties was made in each instance. None of the children was placed in a so-called reform institution, but all have been under the supervision of a Clinic worker. At the termination of the treatment period a summary of behavior of each patient was compiled. These reports indicate that 5 of the 7 patients adjusted satisfactorily. One patient who failed to adjust in behavior was the case which did not display accelerated growth under growth hormone therapy.

**The relation of the iodine tolerance to thyroid function.** Watson, E. M. To appear in this Journal.

The results of 91 intravenous iodine tolerance tests performed on 80 patients are reported. Six hours after the injection of Lugol's solution, representing 250 γ of iodine per kg. of body weight, 18 of 24 individuals without obvious thyroid disease retained 9 to 23% of the injected iodine in the blood stream. The general average for this control group was 18%. In more than one-half the number of patients with thyrotoxicosis, all of the injected iodine had disappeared from the blood in 6 hours. The greatest amount of iodine retained by an individual of this group was 8%. Repetition of the test after subtotal thyroidectomy showed 10 to 35% of the injected iodine remaining in the blood stream. Persons with non-toxic goiters responded to the iodine tolerance test in the same manner as the control cases. Patients with hypothyroidism showed an average of 39% of the injected iodine in the blood after 6 hours, while patients with hypometabolism without clinical signs of hypothyroidism only averaged 15% retention. The iodine tolerance test in conjunction with existing methods of clinical investigation may permit a more comprehensive classification of endocrine disorders.

## ADRENALS

**A quantitative investigation of the water and salt tolerance in Addison's diseases and a consideration of the effectiveness of cortical extracts (Quantitative Untersuchungen**

**über den Wasser-und Salzhaushalt bei Morbus Addisonkranken unter Brücksichtigung der Wirksamkeitsbestimmung von Extrakten der Nebennierenrinde.)** Borst, J. G. S., and J. J. Viersma, Acta med. Scandinav. **91**:127. 1937.

In two patients the urea, water, chloride and sodium excretion was determined in the 24-hour urine samples, and the level in the blood plasma and cells of the chlorides and bicarbonate. The blood pressure and cell volume were also recorded. Alternate periods with and without cortin medication were seen, and NaCl and NaH $CO_3$ were given daily in capsules. No relation was found between the degree of adrenal insufficiency and the blood pressure; cortical extracts had no influence on blood pressure or blood urea. The extracts raised the chloride and bicarbonate content of the plasma, causing chloride, sodium, and water retention, and a rapid decrease in the cell volume of the blood.—J. M. L.

**Involution of the adrenal glands in newly born infants. A biochemical inquiry into its physiological significance.** Bruch, H., and D. J. McCune, Amer. J. Dis. Child. **52**:863. 1936.

Determination of the sodium content and the specific gravity (protein content) of the plasma, the cell volume, and the non-protein nitrogen content of the whole blood, and the amount of total base in the plasma were made in 7 samples of blood from the umbilical vein, and in 106 specimens of venous blood obtained from 72 healthy infants within the first 3 weeks of life. The concentration of sodium and of total base in the plasma remained normal and constant during the period of study, at levels identical with those found in healthy children and adults. The concentration of non-protein nitrogen was inconspicuously elevated during the first few days of life, but at no time was it high enough to suggest adrenal cortical insufficiency. The authors state they have been unable to demonstrate, in newly born infants during the period of adrenal cortical involution, biochemical changes of the sort found in Addison's disease or in adrenalectomized animals.—M. B. G.

**Sympathetic ganglion cell changes in adrenalectomized animals.** Burns, B. I., J. D. Reese and A. H. Sellmann, Proc. Soc. Exper. Biol. & Med. **36**:261. 1937.

In cats with acute adrenal insufficiency, the cells of the cervical and first thoracic sympathetic ganglia 3 to 8 days after adrenalectomy showed marked degenerative changes. These changes were not found in the ganglia of animals suffering incomplete operation, or in animals dying of inanition. The coelic ganglion showed similar changes, but to a more marked degree. —M. H. F.

**Isolation of androstadienone from the urine of a man with a malignant tumour of the adrenal cortex.** Burrows, H., J. W. Cook, E. M. F. Roe and F. L. Warren, Biochem. J. **31**:950. 1937.

Thirty liters of urine was collected from a man of 34 dying of a recurrent cortical tumour, with signs of feminism. The hydrolyzed urine contained 20 times the normal quantity of estrogenic substances (probably estrone) and displayed moderately high androgenic activity. About 0.4 gm. of a hitherto unknown doubly-unsaturated ketone, $C_{19}H_{26}O$, was isolated through the semicarbazone and identified as androsta-3:5-diene-17-one; it could be reduced to 17-androstanone and androstane, and was prepared artificially by withdrawal of the elements of water from dehydroandrósterone. The substance, which is readily isolated, could not be found in the urine of other cancer patients. It showed no estrogenic activity in ovariectomized mice in doses of 1 mg., but displayed about ¼ of the comb-growth-promoting activity of androsterone, and it did not prolong the survival of adrenalectomized rats. Small amounts of 2 other unidentified ketones, without estrogenic activity, were also isolated.—D. L. T.

**Hair growth in adrenalectomized, and adrenalectomized thyroxin-treated rats.** Butcher, E. O., Am. J. Physiol. **120**:427. 1937.

In adrenalectomized rats, the second growth of hair begins earlier than in normal animals. Administration of thyroxine into adrenalectomized rats induces growth sooner than does adrenalectomy alone. Much larger amounts of thyroxine are required to produce a similar effect in unoperated rats. The accelerated hair growth after adrenalectomy therefore seems to be due to thyroxine. Normally the effect of thyroid is held in check by the adrenals.—F. A. H.

**The isolation of pregnane-3, 17, 20 triol from the urine of women showing the adrenogenital syndrome.** Butler, G. C., and G. F. Marrian, J. Biol. Chem. **119**:565. 1937.

The urines of 2 women showing the adreno-genital syndrome have been shown to contain a new triol, m.p. 243° to 240° of the formula $C_{21}H_{36}O_3$. The material was not found in the urine after the removal of an enlarged adrenal. It yielded a diacetate, m.p. 136.5° on treatment with acetic anhydride. Oxidation with lead tetra-acetate indicated the presence of one glycol group, and acetaldehyde and 3-epihydroxyetio-cholane-17-one were identified as the oxydation products. The triol was therefore pregnane-3,17,20-triol. The crude triol was contaminated with appreciable amounts of pregnandiol.—J. M. L.

**Epinephrin and the blood sugar level.** Coletti, C. J., Jr., Am. J. Physiol. **119**:1. 1937.

The purpose of this work was to establish a blood sugar curve after subcutaneous injection of a definite amount of epinephrin per kg. body weight, with very short time intervals between determinations (5-30 min.). Doses of epinephrin ranged from 0.05-0.4 mg. per kg. Blood sugar determinations were made according to the Somogyi micro-modification of the Schaffer-Hartman method. Animals were fasted 24 hr. before injections were made by the subcutaneous route. The typical curve showed a continued rise in blood sugar up to 2 hr. after injection, the rate of change being greatest during the first 15 min. and becoming progressively less during the remainder of the time. There was a progressive rise in percentage change of blood sugar, as the dose was increased, and no evidence that the limit response had been reached with the dosage used.—K. A. Brownell.

**The sexual variation in carbohydrate metabolism. VIII. The rate of absorption of glucose and of glycogen formation in normal and adrenalectomixed rats.** Deull, H. J. Jr., Lois F. Hallman, Sheilla Murray and L. T. Samuels, J: Biol. Chem. **119**:607. 1937.

The glycogen content of the liver was determined in normal and adrenalectomized rats after initial feeding 0.6 gm. of glucose per 100 gm. body weight, and an additional 0.5 gm./100 gm. at the third, fifth and seventh hours. The residual sugar in the G-I Tract was also determined. There was a sex difference in the extent of glycogen formation in both normal and operated animals, but no difference between the two groups. In both groups, the rate of glucose absorption is significantly higher in the female than in the male. There was no evidence that adrenalectomy decreased the rate of glucose absorption or the formation of glycogen. The adrenal gland is not responsible for the sex difference in carbohydrate metabolisms.—J. M. L.

**The purification of adrenal extracts and isolation of an activator of male sex hormones.** Ehrenstein, M., and S. W. Britton, Am. J. Physiol. **120**:213. 1937.

Extracts containing the active principle of the adrenal cortex are prepared by a method involving several revisions of that previously described. The extracts are of a high and uniform potency, and contain 55 mg. of solids per kg. of extracted gland. A series of precipitations from cold benzene, acetone and petroleum-ether respectively produces a further 4-fold purification, and gives potent extracts which are completely soluble in water. The most potent extract (benzene-soluble, acetone-soluble, petroleum-ether-insoluble) was subjected to a fractionation procedure utilizing the chromatographic adsorption technic, but no increased activity would be detected for the material eluted from the upper adsorption layers. (The two other layers have not yet been assayed.) An inactive nitrogenous compound (m.p. 92-94°) was isolated from the material eluted from the middle adsorption layer. A crystalline material (m.p. 119-123°) was isolated from an inactive fraction; this substance proved to be the acid salt of palmitic acid. The authors attach considerable importance to the isolation of this latter compound, in view of its property of activating male sex hormones.—S. L. Cohen.

**Suprarenal tumor with paroxysmal hypertension.** Evans, V. L., J. Lab. & Clin. Med. **22**:1117. 1937.

The case history and autopsy findings in a girl of 12, who died as the result of an adrenal medullary tumor (pheochromocytoma), were recorded. The patient suffered from paroxysmal hypertension and apparently died in shock while operation was being contemplated.—F. A.

**Effects of cortico-adrenal extracts on growth and sexual activities.** Fitzhugh, O. G., Am. J. Physiol. **118**:677. 1937.

In immature rats, administration of adreno-cortical extract in doses of 0.25 to 5 cc. (Britton and Silvette) induced hypertrophy of uteri and ovaries, measurement of follicles and uterine diameters indicating the effect of a female sex-stimulating factor. However, in the immature male inhibition of testicular growth resulted. Body growth was moreover stimulated, the response being greater in the females. The suppressed estrual cycle of the adrenalectomized rat was re-

stored by the extract, a recovery not necessarily associated with a return to normal body weight. Histologically, the extract brought about re-organization and repair in both ovaries and testes of adrenalectomized animals, a property of the extract which, in the writer's opinion, is more or less specific, i.e., that restoration of normal gonadal structure is not entirely due to a general body restoration. There was no conclusive evidence of estrogenic activity in the extract used.—H. O. Haterius.

**Gonadotropic extract from adrenal cortex (Über die Gonadotrope Wirkung von Nebennierenrindenextrakten).** Hoffman, F., Klin. Wchnschr. **16**:79. 1937.

A water extraction of 35 gm. acetone dried adrenal cortex powder injected into immature rats resulted in a substance producing a 100% increase in ovarian weight (up to 18 mg.), consisting of follicular growth and some theca luteinization. The activity was precipitated by 90% alcohol, and was ultrafilterable. When injected simultaneously with prolan, additive effects were noted.—M. H. F.

**Methods of preparation of a gonadotropic substance from the adrenal cortex (Über die Darstellungsmethoden einer gonadotropen Substanz aus der Nebennierenrinde).** Hoffman, F., Endokrinologie **19**:145. 1937.

The gonadotropic substance of the adrenal cortex is readily soluble in water, is precipitated by acetone and by alcohol, and by tungstic and benzoic acids. It is ultrafilterable through an acetic-collodion membrane, and may be prepared free from protein. It is insoluble in the lipoid solvents. The watery extract withstands heating for 20 min. at 80°C. but not at 100°C.—W. J. A.

**Adrenal cortex and fat resorption (Nebennierenrinde und Fettresorption).** Laszt, L. and F. Verzar, Biochem. Ztschr. **288**:351. 1936.

The authors confirmed their previous result that cortical extract restored fat resorption in adrenalectomized rats. The effect of the extract lasted only some hours, and for maximal effect it had to be given simultaneously with the fat feeding. However, the disturbance of fat resorption occurred only after the second day following adrenalectomy. Cortical extract never increased fat resorption above normal. Flavin phosphoric acid had the same effect on fat resorption as cortical extract.—E. Bozler.

**Influence of adrenalectomy on liver fat as varied by diet and other factors.** MacKay, E. M., Am. J. Physiol. **120**:361. 1937.

Adrenalectomy in the rat reduced the amount of fat found in the liver during fasting, and the amount deposited in the liver following the administration of sodium bicarbonate, injection of epinephrine or feeding of high fat diets. During fasting, the depletion of liver fat accumulated during previous feeding of a cream diet, or a low protein high fat diet, was hastened by removal of the adrenals. Since ketosis, produced by experimental alkalosis or feeding of high fat diets, was also reduced by adrenalectomy, the author suggests that the transportation of fat may be affected indirectly by the influence of the adrenals on carbohydrate metabolism.—H. W. Schultz.

**Serum potassium and sodium as altered by adrenalectomy and nephrectomy.** MacKay, E. M. H. C. Bergman and Lois L. MacKay, Am. J. Physiol. **120**:83. 1937.

Two groups of 20 male rats were operated. The first group, which was nephrectomized showed a survival period averaging 67 hours. The second group, which was nephrectomized and adrenalectomized, survived an average of 37 hours. The blood of nephrectomized rats showed a decreased number of red blood cells, while that of adrenalectomized-nephrectomized rats showed hemoconcentration. Any sodium loss which occurred was accompanied by a normal amount of fluid; the sodium concentrations were essentially normal. A much higher concentration of potassium was found in nephrectomized rats than in similar adrenalectomized rats, or in nephrectomized-adrenalectomized animals, suggesting that some factor besides retention of K is responsible for the shortened survival of adrenalectomized-nephrectomized rats over nephrectomized.—Lena Lewis.

**The effect of adrenalectomy on liver fat in fasting and after the administration of anterior pituitary extracts.** MacKay, E. M., and R. H. Barnes, Am. J. Physiol. **118**:525. 1937.

Determinations of fatty acids and non-saponifiable lipids were carried out on the livers of 4 groups of rats, i.e., normal controls, controls receiving anterior pituitary extract, adrena-

lectomized animals, and adrenalectomized animals receiving APE. The extract was made by the method of Best and Campbell from a commercial pituitary powder. All animals were fasted 4 days before being sacrificed for the fat determinations and treated animals received extract over this period, the males, 3 cc. per day subcutaneously, and the females 5 cc. per day of a weaker extract. Adrenalectomy not only abolished the ketones but prevented the accumulation of fat in the liver so characteristic of fasting rats treated with APE. Removal of the adrenals also reduced the amount of fat deposited in the liver during fasting.—K. A. Brownell.

**The liberation of adrenin and sympathin induced by stimulation of the hypothalamus.** Magoun, H. W., S. W. Ranson and A. Hetherington, Am. J. Physiol. 119:615. 1937.

The liberation of circulating sympathin in response to electrical stimulation of the hypothalamus was demonstrated in 4 adrenalectomized cats. The effect was observed by recording the contraction of the chronically denervated nictitating membrane. In 3 acutely denervated animals, the effect, obtained only so long as the adrenals were intact, was therefore ascribed to liberation of adrenin. The prolonged latent period and delayed maximal response is interpreted as meaning that the release of sympathin and adrenin are not responsible for the initial effects of hypothalamic stimulation, but that the direct nervous effects are supplemented and amplified by the circulating hormones.—R. R. Durant.

## ENDOCRINE GENERAL

**The chromatophorotropic hormone of the crustacea: standardization, properties and physiology of the eye-stalk glands.** Abramowitz, A. A., Biol. Bull. 72:344. 1937.

Extirpation of the eye-stalks of the fiddler crab, Uca, produces the pale phase of color change. This is due to melanophore contraction, as the eye-stalks contain endocrine glands. Eye-stalks were extracted with sea water so that the concentration varied from 0.03 to 5 eye-stalks per cc. Experiments performed on 150 specimens of Uca pugilator, which had been blinded by removal of eye-stalks, show that 0.05 cc. of an extract of 1 eye-stalk per cc. contains one Uca unit. The eye-stalk hormone is soluble in water and absolute alcohol, but is insoluble in other organic solvents. It is thermo-stable, destroyed by oxidation and stable in 10% HCl or NaOH for short periods. A protein-free precipitate, obtained by adding ether to an alcoholic solution, contained 2 Uca units per mg. The hormone content of the eye-stalks of crustaceans varied from 0.25 to 5 Uca units.—E. L.

**The influence of endocrine disease on sedimentation rate (Reaksija Osedanija Eritrotsitov pri endokrinnyjkh zabolevanijakh).** Gargala, M. S., Acta Endocrinologia (Klinika Endokrinnyjkh Zabolevanii) 9:234. 1936.

Increased sedimentation rates were found both in myxedema (7 cases) and in exophthalmic goiter (24 cases). A rate of 9 to 15 mm. per hour was considered within the upper limit of normalcy. In myxedema all values ranged between 25 mm. and 45 mm. and appeared on the whole higher than in the exophthalmic goiter. In the latter condition a parallelism between increased sedimentation rate and basal metabolism was found. Although complicating infections were present in some instances, these did not produce the higher values. An elevation of sedimentation rate was also found in diabetes insipidus and diabetes mellitus. Highest values were obtained in some of the latter group, where the incidence of complicating infections was high.—B. N. Toger.

**Some effects of intraperitoneal glucose injections and excess water in normal, adrenalectomized rats.** Gaunt, R., J. W. Remington and Malvina Schweizer, Am. J. Physiol. 120:532. 1937.

Studies were carried out on 32 normal, 44 adrenalectomized and 36 hypophysectomized rats to determine the initial response to and ultimate effect after 12 hr. of glucose injected into the peritoneal cavity. Similar studies were carried out following water intoxication in 16 normal, 7 adrenalectomized and 16 hypophysectomized rats. .1 cc. per gm. body weight of 5.5% glucose was injected intraperitoneally. Hemoconcentration was determined by hematocrit readings. The remaining ascitic fluid after a 12 hr. interval was measured. Adrenalectomized animals showed initial hemoconcentration twice as great as that of the normal, due apparently to shift of water into the tissues. The hemoconcentration was paralleled by nearly complete anuria. The fluid removed from the peritoneal cavity passed into the cells instead of into the blood stream. Adrenal cortical extract in saline largely protected these animals against glucose injections. Hypophysectomized rats showed a delayed hemoconcentration, but ultimately as great as the

adrenalectomized rats. The injected fluid could not be absorbed from the peritoneal cavity. Adrenalectomized and hypophysectomized rats were equally susceptible to water intoxications. In both cases most of the ingested fluid was shifted into the cells. In both cases the susceptibility to water intoxication was probably due to lack of adrenal cortical extract, as injections of cortin protected both types of animals.—L. A. Lewis.

**The relation of endocrine feeding to regeneration, growth and egg capsule production in Planaria maculata.** Goldsmith, E. D., Biol. Bull. **73**:227. 1937.

Planaria maculata, fed on exclusive diets of beef liver, anterior lobe of the pituitary and thyroid gland, showed no difference in head regeneration following amputation of the anterior region. The feeding of aqueous extracts of these organs and starvation showed slower regeneration. The thyroid-fed animals increased in size more slowly than either the pituitary or liver-fed forms. Egg capsule-production was greatest in the liver-fed individuals with the starved or extract-fed individuals producing no capsules.—E. L.

**On the preparation and biological assay of enterogastrone.** Gray, J. S., W. B. Bradley and A. C. Ivy, Am. J. Physiol. **118**:463. 1937.

A more efficient and dependable method is described for preparing, from the duodenal mucosa of hogs, large amounts of an extract which inhibits gastric motility and secretion. The extract enterogastrone was assayed by testing its effect on hunger and postprandial contractions of the stomach in trained dogs. The inhibitory effect, found to be specific, was the same on hunger contractions as on motility, due to distention of the stomach. The effect on gastric secretion was assayed on dogs with vagotomized pouches of the entire stomach, in which a constant rate of secretion was obtained by repeated injection of histamine. The inhibitory effect was found to depend on the rate of flow of gastric juice before injection, and is more marked on the concentration of hydrochloric acid than on the volume of secretion. Enterogastrone demonstrates a different type of action on motor and secretory functions. Small doses completely inhibit motility for a period proportional to the dose, whereas they merely lower the secretory rate to a constant, reduced level which persists for several hours.—R. R. Durant.

**Comparison of responses of smooth muscle to d1-B-(3, 4-dihydroxyphenyl) -B-hydroxyethylamine (arterenol), 1-epinephrine, and "liver sympathin." I. Responses of smooth muscle of iris and of vascular smooth muscle.** Greer, C. M., J. O. Pinkston, J. H. Baxter, Jr. and E. S. Brannon, J. Pharmacol. & Exper. Therap. **60**:108. 1937.

The vasomotor and pupillary responses produced with arterenol are comparable to those produced by "liver sympathin."—E. L.

**Studies on the sensitivity of smooth musculature to exogenous epinephrine.** Heinbecker, P., Am. J. Physiol. **120**:401. 1937.

Sensitization of the iris of the cat was produced by removal of the superior cervical ganglion. The sensitivity as tested by subcutaneous injections rose to a maximum in 8 to 12 days. The sensitivity of sensitized smooth muscle is still further increased by thyroid feeding and hypophysectomy, whereas normal muscle is only slightly affected. The sensitivity is diminished by thyroidectomy, gonadectomy and adrenalectomy. By perfusion experiments the author showed that removal of the abdominal sympathetic trunk sensitized the blood vessels of the leg to adrenaline, but not to pituitrin. Histological studies did not reveal any difference between normal and sensitized smooth muscle. Because activity decreased the sensitivity to adrenaline in normal muscle the author inclines to the view that sensitization by denervation is due to the accumulation of a store of adrenaline, or of a substance on which it acts.—E. Bozler.

**A case of von Gierke's Disease with marked lipemia.** Hogg, P., and J. B. Sidbury, J. Pediat. **10**:798. 1937.

A case of von Gierke's disease is reported in a 16-months' old boy. The principal findings were hepatomegaly, hypoglycemia, acetonuria, aglycogenolysis and marked lipemia.—M. B. G.

**Relation between basal metabolism and adolescent growth.** Lewis, C. A., Amer. J. Dis. Child. **51**:1014. 1936.

The relationship between the basal metabolic rate and pubertal acceleration was studied at an orphanage in 39 postpubertal girls between 14 and 16 years of age. No relationship was found between physiologic age or the rate of growth and the B.M.R.—M. B. G.

**Relationship of basal metabolism to dietary intake.** Johnston, J. A., and J. W. Maroney, Am. J. Dis. Child. **51**:1038. 1936.

A study extending over 3 years was made of 33 children, who were maintained on measured diets for periods averaging 945 months each. The ages ranged from 4 to 15 years. The diets were begun at submaintenance levels, and intake increased to a point where increased height, weight and positive nitrogen balance were obtained; lowering of the basal heat production was evident. While the basal metabolism can in most instances be brought from subnormal to normal levels, only slight increases beyond normal are evident, although such increases, calculated on the basis of the surface area of the body, are possible with a high protein intake. Maximal elevation of the B.M.R. is a result of a high intake of calories and a high intake of protein. The addition to an adequate diet of large amounts of carbohydrates was followed in one instance by a slight decrease in the basal metabolism. The addition to an adequate diet of large amounts of fat was followed by a slight increase in the basal metabolism. The addition to an adequate diet of a high increment of protein was followed by a marked increase in the basal metabolism.—M. B. G.

**Growth and basal metabolism. V. Basal metabolism of high school children.** Nakagawa, I., Amer. J. Dis. Child. **53**:985. 1937.

The basal heat production of 23 Japanese high-school children (10 boys and 13 girls between the ages of 12 and 15 years) was found to be low as compared with the Talbot standards.—M. B. G.

**Experiments on hormonal interruption of broodiness in hens (Versuche über Hormonale Beeinflussung der Brütigkeit bei Hennen).** Podhradský, J., Č. S. R. Brno and K. Wodzicki, Arch. f. geflügelkunde **9**:123. 1935.

Prolan A, thyroid and pituitrin were administered to 8 brooding Rhode Island red hens over periods of 20 days. There was no interruption of broodiness over this period or 7 days afterwards with any of the hormones. In the group receiving thyroid, no moulting was produced, although the same dosage produced prompt and heavy moulting in non-brooding hens.—M. O. L.

**Etiology of mongolism with special reference to its occurrence in twins.** Rosanoff, A. J., and L. M. Handy, Am. J. Dis. Child. **48**:764. 1934.

The authors enter into a comprehensive discussion as to the bearing of age, sex and maternal condition on the pathogenesis of mongolism. They think that damage to the ovary is responsible for the condition, and propose that systemic studies of ovaries of mothers with mongolian idiots be made. They postulate that foci of tissue change, perhaps scars marking the site of old ovulation, are the cause of mongolism. The ovaries of any girl who has menstruated one or more times contain one or more foci. The chance of producing a child with mongolism increases with increasing age. He presents data on all reported cases of mongolism in twins—64, including 5 of his own.—M. B. G.

**Basal energy metabolism and creatinine in the urine. I. Observations in children.** Talbot, N. B., Am. J. Dis. Child. **52**:16, 1936.

Palmer, Means and Gamble showed the possibility of utilizing the excretion of creatinin as a basis for the evaluation of basal energy metabolism. The author having found a close relationship between it and heat production, claims that this method is more accurately informative.—M. B. G.

**On the mechanism of stimulation of carotid gland chemoreceptors.** Winders, C. V., Am. J. Physiol. **118**:389. 1937.

A study of anoxic, hypercapnic and asphyxic stimulation of the carotid gland before and after poisoning with mono-iodoacetic acid leads to the following observations. There is a progressive increase in ventilation of the animal during the onset of poisoning of the carotid segments, apparently due to an action at the carotid gland. Concentrations of the poison which abolish the reflex anoxic augmentation of ventilation, or convert it to a late depression, may affect the pressoreceptor reflexes very little. Under certain conditions the anoxic response may be thus abolished or converted, while the hypercapnic response persists strongly. Hypercapnia appears able to stimulate the poisoned receptors under a degree of anoxia, which by itself is incapable of stimulating. It is concluded that anoxic stimulation of chemoreceptors of the carotid gland is dependent upon glycolytic processes, and that hypercapnic stimulation may be

at least largely independent of such processes; that glycolysis is probably an actual link in setting up the local excitation by anoxia, rather than merely a necessary source of energy during the anoxia; and that the required part of that link is to be found in the formation of the intermediary or final acid products of glycolysis.—A. J. Derbyshire.

## GONADS

**A study of the hormonal content of ovarian cyst fluids.** Adair, F. L., and R. M. Watts, Am. J. Obst. & Gynec. 34:799. 1937.

Examination of 229 fluids from ovarian and other genital cysts shows estrogenic substances in significant amounts in certain members of almost all the subgroups of ovarian, but rarely in non-ovarian cysts. No correlations with the menstrual cycles could be established. In 21 cases, search for gonadotropic hormone was made, but usually none was found.—E. L. S.

**Relation between the anterior pituitary, adrenal, and luteinisation (Beziehungen swischen Hypophysenvorderlappen, Nebenniere, und Luteinisierung).** Balint, J., Klin. Wchnschr. 16:136. 1937.

The injection of multiple doses of 5 to 10 R.U. of prolan into adrenalectomized infantile rats did not produce corpora lutea, although the injection of similar amounts of APE did so. Moreover, the injection of a single dose of 10,000 U of estrin into adrenalectomized infantile rats, just as in the intact animal, led to corpora-lutea formation.—M. H. F.

**The action of the mammary gland upon the ovary.** Cherry, T. H., J. Lab. & Clin. Med. 22:711. 1937.

The experiments were designed to show whether the lactating breast exerts any inhibitory effect on follicle maturation as suggested by the fact that lactating women are less apt to become pregnant. It was found that mammary extracts inhibited the action of APL on the immature rat's ovary, and on the rabbit ovary. Furthermore, the preparation was tried with benefit on 50 women with functional uterine bleeding, on the basis that they were suffering from hyperestrinism.—F. A.

**Superfecundity in rats treated with mare gonadotropic serum.** Cole, H. H., Am. J. Physiol. 119:704. 1937.

Mare serum, assayed at 50 R.U. per cc. was prepared and utilized in experiments upon the effects of various dosages on the fecundity of mature and immature rats. In 155 mature animals, dosages of 3 to 24 R.U. were given during di-estrum, estrus and metestrum. Superovulation was not induced, although the average litter size was increased, but this did not exceed in size the largest litters found in control animals. Excessive doses reduced the percentage of fertile matings both in adult and in young animals. Failure of marked increase in litter size in mature animals was attributed to failure to induce superovulation. The length of cycles was definitely shortened (from 4.48 to 3.91 days). Superovulation and superfetation (as many as 28 fetuses) occurred in rats injected on the 22nd day of life, and mated on the 25th day. 49% of rats injected on the 26th to 30th day of life mated. Of rats sacrificed on the 10th to 12th day, 38% had more than 20 fetuses; one rat, sacrificed on the 17th day, had 26 normal-appearing fetuses; one on the 21st day had 23 living fetuses. The largest number born was 17. Failure of mature animals to display superfecundity, i.e. the ability to implant an abnormally large number of fetuses, lay apparently in a failure to liberate an excessive number of ova. The regulating mechanism, apparently lacking in the immature but present in the adult, remains obscure.—H. O. Haterius.

**The effect of estrin upon the basal metabolism rate and the nervous symptoms of ovariectomized women.** Collett, M. E., J. T. Smith, and G. E. Wertenberger, Am. J. Obst. & Gynec. 34:639. 1937.

Selected patients with ovariectomy were studied before therapy with sufficient basal tests to secure very uniform levels. The use of estrogenic substances orally and parenterally was carried out for varying periods. The initial basal rates tended to be 12 to 20% below Harris-Benedict predictions, and the therapy frequently led to increased metabolism. The results are not seen immediately, but may appear during or after a period of sustained use of the hormones. The immediate effects are instability of the metabolism from day to day, with varying clinical benefit. The therapy achieved different degrees of control of the common vasomotor and psychic symptoms. There was no parallelism between the clinical benefit and the change in metabolic rates.—E. L. S.

**Follicular growth rate and ovulation after various experimental procedures in the guinea pig.** Dempsey, E. W., Am. J. Physiol. **120**:126. 1937.

Determinations of the follicular volumes and follicular growth rates were made with respect to the effects of hypophysectomy, pregnancy, removal of corpora lutea, estrin and progesterone administration. Of the procedures employed for abolishing follicular growth, hypophysectomy proved the only effective method. Pregnancy, ablation of corpora lutea, injections of progesterone (0.05 I.U. daily for 20 days, beginning on 10th day of cycle), estrin (progynon-B, Schering, in dosages of 250 I.U. daily for 20 days from the 10th day of the cycle) and male sex hormone (testosterone and androsterone benzoate, 0.1 mg. daily for the 14th day of the cycle to the beginning of the next estrus) produced no alteration of the basic growth rate. The observation that pregnancy and injections of progesterone prevent pre-ovulatory swelling and ovulation is attributed to direct inhibition of LH release from the pituitary, since simultaneous injections of the latter substance induce ovulation. The precocious ovulation which ensues upon ablation of the corpora lutea is accomplished by the initiation of ovulatory changes before follicles have attained full normal size. The fact that estrin failed to inhibit the cyclic follicular growth, although it interfered somewhat with ovulation, leads the author to question the validity of current theories postulating a rising tide of estrin as inhibiting the continued production of FSH and causing the timely release of LH. It suggests rather that FSH secretion occurs at a fairly constant rate during the cycle. Periodic release of LH may therefore be accomplished by a rhythm inherent in the pituitary, or in consequence of a more central control.—H. O. Haterius.

**A study of the augmentation of ovarian weights as affected by zinc sulphate, antuitrin-S and thyroid implants.** Emery, F. E., Am. J. Physiol. **118**:316. 1937.

The intraperitoneal administration of P.U. extract (antuitrin-S) and zinc sulphate produced ovarian weights in immature rats significantly in excess of those evoked by antuitrin-S alone. However, this augmentation was lacking when the zinc sulphate was given subcutaneously. Merely doubling the dosage of antuitrin-S (to 100 R.U.) elicited a slight increase in weights, whereas doubling the amount of implanted pituitary tissue proved more effective. Greatest weight increments were obtained by the administration of antuitrin-S and pituitary implants in combination. Neither protein (thyroid tissue) nor zinc sulphate augmented the effects of pituitary implants. Delayed absorption may be invoked as a factor in augmentation, although the author is of the opinion that other factors, as yet not clear, may perhaps contribute.—H. O. Haterius.

**Effects of ovariectomy on body growth and organ weights of the young albino rat.** Freudenberger, C. B., and P. M. Howard, Proc. Soc. Exper. Biol. & Med. **36**:144. 1937.

In rats spayed at the age of 26 days and autopsied at 3 months, the body weight and length and the weights of the endocrine organs were greater than in control animals autopsied at the same time.—M. H.F.

**The effects of hysterectomy on mammary gland development in a rabbit.** Gillard, J. L., Am. J. Physiol. **120**:300. 1937.

Hysterectomized animals were rendered pseudopregnant, and biopsy material removed at the time of ovulation and every 3 or 4 days thereafter during the course of pseudopregnancy. Pseudopregnancy persisted 25 days, as contrasted with an 18-day period in non-hysterectomized animals. Only duct formation occurred prior to ovulation and corpus luteum development, a reaction attributed to estrin influence. Marked lobule formation was evident by the third day following follicular rupture, probably as a result of progestin stimulation. Moreover, prolonged estrin treatment in a spayed virgin female induced only duct development. Although it nearly doubled the life of the corpora lutea, hysterectomy did not lead to mammary development in excess of that found in the intact pseudopregnant animal. Hysterectomy did not prevent subsequent appearance of estrus.—H. O. Haterius.

**On the relation between the production of oestrine during pregnancy and the sex of the foetus and the size of the placenta (Ueber die Abhangigkeit der Oestronproduktion wahrend der Schwangerschaft von dem Geschlecht der Frucht und von der Grosse der Placenta).** Gram, L., Biochem. Ztschr. **289**:397. 1937.

Pregnancy urine was assayed according to Zondek. The secretion of estrin was the same in 10 women, who gave birth to girls, as 8 who had boys. Also the estrin content of the placenta of both sexes was the same. The problem was studied because of its significance in the problem of determining the sex of the embryo.—E. Bozler.

**Prostatic type of paraurethral glands induced in female rats by administration of male sex hormone.** Hamilton, J. B., and J. M. Wolfe, Proc. Soc. Exper. Biol. & Med. **36**:465. 1937.

In untreated female rats, grossly visible paraurethral glands (Skene's ducts) were noted in only 9.4%. In mature or immature females receiving 500 gamma of male hormone, the incidence was about 60%, regardless of whether the male hormone was given alone, or together with an equal amount of estrin. The incidence in spayed rats with or without estrone was nil. The histology of the structures closely resembled that of the male prostate.—M. H. F.

**Positive Friedman test in the pregnant rhesus monkey.** Hamlett, G. W. D., Am. J. Physiol. **118**:664. 1937.

In view of the almost uniform failure of the Friedman test in subhuman species, the possibility was put to test that, in the macaque at least the urinary excretion of prolan might be a transitory process. Tests made as early as possible during pregnancy gave typical positive reactions for a period of about one week following implantation, after which time the urine became negative. This observation probably accounts for the failure of previous investigators. Positive reactions were obtained only during the 19th to the 25th days postovulation and conception. Tests prior to the 15th day proved negative.—H. O. Haterius.

**The retrogression of the lactating mammary gland in the guinea pig.** Hesselberg, Cora, and L. Loeb, Am. J. Physiol. **118**:528. 1937.

Since the earlier finding of Kuramitsu and Loeb that an occluded gland no longer secreted, which finding resulted in their conclusion that the continuance of lactation is a local effect resulting from withdrawal of milk, failure of confirmation in other species has been reported. In these species (e.g., the rat) reflex control was inferred. In the present work, a series of 25 pregnant guinea pigs was studied. Ten were allowed to suckle their young postpartum; 4 were deprived of their young at birth; in 11 one nipple was ligated at the time of parturition, leaving one side undisturbed. Findings were based upon subsequent microscopic examination of removed tissue. Ligation of one breast following upon parturition resulted in rapid retrogressive changes, although the opposite gland continued to secrete, if suckling on that side were allowed to continue. In confirmation of earlier findings in the guinea pig, secretion and retrogression are believed to be controlled by local mechanisms. The authors are of the opinion that species differences may account for the disparity in results obtained in the rat.—H. O. Haterius.

**Effects of progesterone on the female genital tract after castration atrophy.** Hisaw, L., R. O. Greep and H. L. Fevold, Proc. Soc. Exper. Biol. & Med. **36**:840. 1937.

The daily injection of 4 R.U. of synthetic progesterone given a castrate monkey for 18 days led to vaginal proliferation, development of typical sexual skin coloration, growth of the uterus, with the development of premenstrual endometrium and the growth of characteristic epithelial proliferations around a thread placed in situ at laparotomy on the 11th day of treatment. The monkey had been castrated 242 days earlier. Progestational proliferation was also obtained in castrate rabbits with 6 RB.U. daily, and some uterine and vaginal growth obtained in castrate rats with 1.0 mg. daily for 6 days.—M. H. F.

**The luteinizing influence of oestrin through the luteinizing hormone of the anterior pituitary (Über die luteinisierende wirkung des follikelhormons durch beeinflussung per luteogenen hypophysenvorderlappensekretion).** Hohlweg, W., and A. Chamono, Klin. Wchnschr. **16**:196. 1937.

A single subcutaneous injection of 50 U of estradiol benzoate given 50 gm. rats (about 8 weeks old) led to corpus-luteum formation as early as 4 to 5 days later. Hypophysectomy, before the injection or 2 days after the injection, prevented the reaction, but reaction was not hindered by hypophysectomy 4 days after the estrin injection, the animals being sacrificed 4 days later (8 days after the injection). Moreover, the operation at this time did not apparently interfere with the development of the corpora. It is concluded from these results that the luteinizing hormone is released 2 to 4 days after the stimulus of estrin.—M. H. F.

**Adrenalectomy in mice, and the replacement of X zone bearing adrenals by cortical extract with especial reference to adrenalgonad relationships.** Howard, Evelyn, Am. J. Physiol. **120**:36. 1937.

Of 34 adrenalectomized 3-week old male mice, 18 operated without ligation died within 12 days. Of 16, in which the adrenal vessels were ligated, 5 survived indefinitely, the re-

mainder dying within 10 days. Hence, young mice are no exception to the rule that complete adrenalectomy is fatal. The capacity of extract of non-X-bearing adrenals (pig) to replace glands possessing an X zone (mice) was determined as follows: 21 day-old female mice were bilaterally adrenalectomized and treated orally with an excess of the test extract (charcoal adsorbate). Life was maintained, and bodily growth was continued at essentially a normal rate. Therefore surgical removal of the X zone need not be compensated by an X zone extract in order to maintain life and growth. Further, no appreciable repressive action could be detected, either of the extracts or of the X zone, so far as the normal maturation of the reproductive system was concerned.—H. Haterius.

**Sexual and asexual reproduction in Euplanaria tigrina (Girard).** Kenk, R., Biol. Bull. **73**:280. 1937.

In Euplanaria tigrina, collected at 4 different localities, 3 showed a race propagating asexually and one propagating sexually. The asexual race reproduces exclusively by fission, whereas the sexual race propagates sexually during the colder season and asexually during the warmer season.—E. L.

**Studies relating to time of human ovulation. II. During lactation.** Kurzrok, R., P. M. Lazz and Jane Smelser, Proc. Soc. Exper. Biol. & Med. **36**:356. 1937.

Suction endometrial biopsies (125) were obtained from 30 patients in whom there appeared regular catamenia during lactation. The biopsies were obtained during the last 10 days of the cycle, and ovulation was assumed to have taken place if a premenstrual endometrium was found. A proliferative endometrium was assumed to indicate an anovulatory cycle. Of the 106 cycles investigated, 93 were ovulatory, 45 anovulatory, and 22 doubtful.—M. H. F.

**Prostatic hypertrophy. Studies of hormonal treatment with testosterone salts (L'hypertrophie de la prostate. Essais de traitement hormonal par les sels de testostérone).** Laroche, G., F. Marsan, E. Bompard and A. Corcos. Presse méd. **45**:932. 1937.

The treatment consisted of 2 or 3 series of 12 injections, giving a total of 1 to 2 gm. of testosterone during 6 months. The results reported were: relief from frequency of urination in simple prostatism, improvement in 50% of the cases of incomplete retention and re-establishment of voluntary micturition in 72% of the cases of complete retention.—A. E. Meyer.

**Effect of progesterone on cell-division in theuterine epithelium.** Lloyd, C. W., Proc. Soc. Exper. Biol. & Med. **36**:190. 1937.

Mitotic figures were counted in uterine mucosa of spayed rabbits which had been injected for 3 days with 0.5 mg. of progesterone, or with 50 I.U. of estrin, and then autopsied on the fourth day after the operation. The number of mitotic figures in the estrin-treated animals was not materially different from the number in untreated controls, but the number in the progesterone-treated rabbits was increased 50 times.—M. H. F.

**Effect of prolonged administration of oestrone on chemical composition of bone (Influencia de la administración prolongada de estrona sobre la composición química del hueso).** Macola, B. A., Rev. Soc. argent. de biol. **13**:105. 1937.

Young white rats (17 animals weighing from 15-90 gm.) received 12 to 40 U estrone in oil subcutaneously daily for 2 to 3 months; 5 controls were injected with the oil only. Total ash, organic substances, Ca and Mg showed no difference in the treated and control groups. Total P and inorganic P were significantly decreased in the treated group.—J. T. L.

**The effect of progestin and progesterone on ovulation in the rabbit.** Makepeace, A. W., G. L. Weinstein and M. H. Friedman, Am. J. Physiol. **119**:512. 1937.

To determine the possible ovulation-inhibiting action in the rabbit, several series of postpartum animals were injected with synthetic progesterone or with a purified preparation of progestin. In the first series, 14 animals were given either progestin or progesterone in doses of 2 Rb.U. (Corner-Allen); or 2 mg. daily for 5 days. At the end of the fifth day, only 5 accepted the male. The mated rabbits failed to ovulate; the animals refusing to mate were given 1 M.O.D. of a P.U. extract (antuitrin-S), as a result of which 7 ovulated. A second group of 7 received 1 mg. progesterone daily for 5 days. Three accepted the buck but failed to ovulate. Of the 4 unmated animals, 2 received no further treatment, and subsequent laparotomy revealed no evidence of gonadal stimulation. The remaining 2 were injected with 1 M.O.D.

of P.U. extract; one ovulated and one displayed cystic follicles. A third series of 5 received 5 mg. progesterone per day for 5 days. One was mated, with no resultant ovulation. The remaining 4 received 1 M.O.D. of P.U. extract, as a result of which 2 ovulated, and 2 did not. In a final series, 5 animals received 2 mg. progesterone per day for 5 days. None accepted the male, neither on the 5th nor on the sixth day, but did so on the seventh. Ovulation occurred in each instance. It is apparent, therefore, that both extract of sow's corpora lutea and synthetic progesterone inhibit post-coitum ovulation in the rabbit. The inhibition, however, does not persist longer than 48 hours after the final injection. Further, despite administration of doses equal to or greater than the amount required to sustain pregnancy, the ovarian follicles will still ovulate as a result of P.U. extract stimulation. This action interpreted means that the inhibition of ovulation during pregnancy and pseudopregnancy is not through direct action upon the follicles, but involves a more central mechanism.—H. O. Haterius.

**Pseudomenstruation in the human female.** Mazer, C., S. L. Israel and L. Kacher, Surg. Gynec. & Obst. **65**:30. 1937.

The authors are of the opinion that the terms pseudomenstruation and anovular menstruation are not synonymous. When the diagnosis is based on endometrial findings alone, the term pseudomenstruation is preferable, because the presence of anovular menstruation cannot be proved without the concomitant study of the ovaries. The etiology of pseudomenstruation is a failure of ovulation (an ovulatory menstruation), and an inherent or quantitative lack of uterine responsiveness, or quantitative disparity in the production of the two ovarian hormones. Thus, in a study of 68 cases (normal women) ranging from 22 to 47 years of age, with a group average of 3 children, and with regular menses (29.8 days) only one quadripara showed the phenomena of pseudomenstruation. Whereas, in a collateral study of 65 women suffering from functional sterility, with no evidence of the secretory phase, 19 of the 65 specimens studied, or about 30% showed evidences of pseudomenstruation.—L. B. S.

**Effect of testosterone on somatic growth.** McEuen, C. S., H. Selye and J. B. Collip, Proc. Soc. Exper. Biol. & Med. **36**:390. 1937.

The daily injection of 2 mg. of testosterone in oil given rats for 23 days led to diminished size of the gonads of both sexes, anestrus in the injected females, and development of the mammary glands. There was no influence on somatic growth, and no influence on the size of the pituitary. In this respect the results differ from those obtained with estrin injections.—M. H. F.

**The effect of oestrone on the secretion of the gonadotropic complex as evidenced in parabiotic rats.** Meyer, R. K., and R. Hertz, Am. J. Physiol. **120**:232. 1937.

A quantitative determination was made of the effect of estrone upon the amount of gonadotropic secretion of castrated male and female rats in parabiotic union with normal females. Daily estrone injections ranging in quantity from 0.05 gamma to 5 gamma were made within a few hours of operations. 0.20 gamma of estrone per day prevented ovarian hypertrophy in one series, regardless of sex of the castrate parabionts. Reduction of dosage to 0.05 gamma per day did not entirely prevent hypertrophy in the castrated male-female pairs. A statistically significant sex difference was noted in the pairs of another series; injected with 0.20 gamma per day, ovarian hypertrophy was prevented in the castrated female-female pair, and decreased in castrated male-female parabionts. This difference in degree of ovarian hypertrophy is believed to be due to the greater potency of the castrate male gland. The degree of ovarian response increased in inverse ratio to the dosage of estrone. The authors believe the amount of ovarian hypertrophy obtained to be a measure of the quantity of gonadotropic complex secreted by the pituitary, and further that the method of parabiosis is an effective one for studying quantitative variability, providing the number of pairs used is sufficient for statistical treatment.—H. O. Haterius.

## HYPOPHYSIS

**Action of carbohydrate metabolism hormone of the anterior hypophysis on blood sugar (Über die Wirkung des Kohlehydratstoffwechselhormones des Hypophysenvorderlappens auf den Blutzucker).** Anselmino, S. J., Endokrinologie **19**:30. 1937.

The problem was to determine whether the carbohydrate metabolism hormone of the anterior pituitary exerts an effect upon the blood sugar of the rat similar to its marked action in reducing the glycogen content of the rat's liver. Since it has not been possible to separate this hormone from other substances which act on the blood sugar in gland extracts, the hormone was

prepared from blood serum. It was found that no appreciable effect on the blood sugar could be detected, although the hormone reduced the liver glycogen of the same animals to less than 10% of normal values.—W. J. A.

**Effects of administering adrenotropic extract to hypophysectomized and thyroidectomized tadpoles.** Atwell, W. J., Am. J. Physiol. **118**:452. 1937.

In view of reports that APE induce adrenocortical hypertrophy, experiments were designed to determine the possible rôle of the thyroid as an intermediary agent. Using an adrenotropic extract (Collip), both hypophysectomized tadpoles and hypophysectomized-thyroidectomized tadpoles (R. pipiens) were given intraperitoneal injections of 0.05 cc. per day for 20 injections following upon a postoperative interval of 5½ to 6 months. The thyroids of the first group were essentially normal, in contrast to untreated controls, demonstrating some degree of thyreotropic activity of the extract employed. In the second group, with both hypophyses and thyroids absent, the adrenals were large, with abundant osmiophilic material, and compared favorably with those of animals not thyroidectomized prior to treatment. Absence of the thyroid therefore failed to prevent the repair or even the hypertrophy of the atrophied adrenal glands. Nor, on the other hand, did possession of thyroid fragments in some of the animals increase the degree of adrenal response. Moreover, there was no compensatory hypertrophy of thyroid fragments inadvertently left in situ, at the time of thyroidectomy.—H. O. Haterius.

**Estrin and calcium metabolism (Follikelhormon und Kalkstoffwechsel).** Bach, E., Klin. Wchnschr. **16**:280. 1937.

On measured amounts of a bread-and-milk diet, the daily injection of large amounts of estrin (30 to 100 γ) into white rats led to an increased blood calcium and decreased calcium content of muscle, bone and skin. The calcium balance was more positive on the first day after beginning injections, but thereafter was markedly negative.—M. H. F.

**Suprarenal gland: therapeutic agent.** Barbour, O., Illinois M. J. **72**:343. 1937.

As a result of experience covering a period of 7 years involving a series of more than 2500 clinical cases, Barbour found whole suprarenal gland useful in the treatment of a number of conditions encountered in general practice. Among these were certain respiratory disorders associated with cough (especially connected with hypoglycemic and vagotonic conditions), vasomotor rhinitis, laryngeal stridor, asthma, selected gastrointestinal conditions (spastic vomiting, infantile colic and diarrhea), idiopathic cyanosis of the new-born, nervous disorders requiring an antispasmodic or a sedative, certain skin disorders (eczema, urticaria and diaphoresis), and as an adjuvant in hypothyroidism. Contraindications to the use of suprarenal substance are circulatory decompensation, nephritis and constipation.—I. B.

**Relation of vitamin E to the anterior lobe of the pituitary gland.** Barrie, M. M. O., Lancet. **233**:251. 1937.

A record of 44 litters of rats born to rats deficient in Vitamin E were compared to a similar number of normal rats. There was evidence that the gonadotropic, glactotropic, and the thyrotropic hormones of the pituitary were impaired. Histological evidence substantiated this, insofar as the basophils and acidophils showed some degree of degranulation. Vitamin E deficient rats grew slowly and were abnormal in appearance. The condition suggests pituitary dwarfism with secondary cretinism. When adequate doses of Vitamin E were administered to these animals there was a return to the normal.—L. B. S.

**The mechanism of the anti-gonad action of prolactin in adult pigeons.** Bates, R. W., O. Riddle and E. L. Lahr, Am. J. Physiol. **119**:610. 1937.

Evidence is adduced to indicate that the anti-gonadal activity of prolactin in adult birds is not the result of direct action upon the gonad, but rather the indirect effect of its action upon the pituitary where, in common with estrone, progesterone, and testosterone, it seems to inhibit FSH release. This is based upon the observation that the anti-gonad action occurs only when the available FSH is below a certain value, a value which presumably approximates that required for testis maintenance. Moreover, in the immature bird, the pituitary of which is said to release no FSH, the gonads are very sensitive to the administration of FSH, even in the presence of prolactin. This observation is believed to be incompatible with the concept of a direct antagonistic action of the two hormones on gonadal tissue. Since the sex hormones exert an adverse influence through suppression of pituitary release, and since their administration to mature birds produces results similar to those following prolactin administration, it is

believed that prolactin acts in a similar manner. Body weight increment was produced by prolactin administration, an increase which could be partly or wholly nullified by preparations containing thyrotropic hormone.—H. O. Haterius.

**The composition of rabbit milk stimulated by the lactogenic hormone.** Bergman, A. J., and C. W. Turner, J. Biol. Chem. **120**:21. 1937.

A study was made of the lactose content of the mammary glands of rabbits in which various degrees of lactation were produced by lactogenic hormone. A correlation was found between the Gardner and Turner rating and the amount of lactose in the gland. The milk produced by the lactogenic hormone showed a higher fat content and a lower ash content. The lactose and total solids were similar. A male rabbit induced to lactate by the hormone raised 3 young to weaning age without further hormone administration.—J. M. L.

**Insulin shock therapy in schizophrenia.** Braceland, F. J., and D. W. Hastings, Pennsylvania M.J. **41**:7. 1937.

Insulin shock treatment was started on 30 patients, all of whom showed schizophrenic symptoms. Treatment was continued on an average of 6 weeks for each patient. Results are reported on 26 cases. Insulin treatment is apparently a valuable weapon against schizophrenia. It should be tried in all early cases, and in any case showing paranoid symptoms. The paranoid patients showed the most favorable results.—I. B.

**Optic neuritis in hyperthyroidism. Report of a case with review of the literature.** Brown, R. B., and G. A. Schwarz, Arch. Int. Med. **60**:668. 1937.

A case of thyrotoxicosis is presented in which optic neuritis was found to be an associated condition. Despite the presence of other possible etiologic factors, because of the parallel fluctuations in the inflammation of the optic nerves and the thyrotoxic state, it is believed that hyperthyroidism was the chief cause of the optic neuritis. It is pointed out that this condition must be rare, and that the mechanism of the relationship is not definitely known.—I. B.

**Pituitary and carbohydrate metabolism (Aperçu sur le rôle de l'hypophyse dans le métabolisme hydrocarboné).** Cahane, M., Presse méd. **45**:550. 1937.

Injections of emulsions of rat pituitaries increased the blood sugar in the frog by 4 to 13 mg. per 100 cc. Pregnancy urine caused a blood sugar increase of 24 to 29 mg. in rats. The hepatic glycogen remained unchanged, whereas the muscular glycogen decreased. Posterior lobe extract produced a blood-sugar drop in the rat concomitant with a decrease in muscle glycogen. Anterior pituitary extract slightly increased the blood sugar in normal humans.—A. E. Meyer.

**Carcinoma of the islands of Langerhans with hypoglycemia and hyperinsulinism.** Cragg, R. W., M. H. Power, and M. C. Lindem, Arch. Int. Med. **60**:88. 1937.

The case described occurred in a married woman of 41, with spontaneous hypoglycemia in which frequent feedings of carbohydrate were required to avert convulsions and coma. The liver appeared to function normally, and to be able to release glycogen as dextrose when epinephrine was injected. At necropsy, carcinoma of the islands of Langerhans was present, with multiple metastases, particularly to the liver. The cells of the metastatic nodules in the liver closely resembled those of normal islands of Langerhans. This view was confirmed by the preparation from the hepatic nodules of an extract which contained insulin, a substance which, according to available evidence, is present in extractable amounts solely in cells of the islands of Langerhans. Classification of the condition as due to true hyperinsulinism seems justified. This is the second case in which the presence of insulin in metastatic carcinoma of the islands of Langerhans has been demonstrated. The previous demonstration of this during study of a similar case by Wilder, Allan, Power and Robertson is fully confirmed, and the evidence is thus materially strengthened that metastatic carcinoma cells of the islands of Langerhans can retain the function of the parent cells.—I. B.

**Increased urinary excretion of iodine in hyperthyroidism.** Curtis, G. M., and I. D. Puppel, Arch. Int. Med. **60**:498. 1937.

There is an increased urinary excretion of iodine in certain cases and phases of hyperthyroidism. The grand average value for urinary iodine in 40 patients investigated over a period of 298 days was 184 micrograms per day, which is between 3 and 4 times greater than the

normal grand average (51 micrograms) for persons living in central Ohio. The grand average daily urinary excretion of iodine in toxic nodular goiter was greater than that in exophthalmic goiter. The variability of the daily urinary excretion of iodine in hyperthyroidism is much greater than that of the normal urinary excretion of iodine. It ranged from 13 to 954 micrograms daily, with averages ranging from 30 to 593 micrograms. The many variations in the urinary excretion of iodine in the same patient and in different patients with hyperthyroidism correspond to the many variations which may occur in any of the other clinical and laboratory features of this disease. An increased urinary excretion of iodine is sometimes observed also in conditions other than clinical hyperthyroidism. Determinations of the blood and urinary iodine are at present of the greatest differential diagnostic importance in certain cases of neurocirculatory asthenia, in which the clinical features simulate borderline hyperthyroidism. Increased urinary excretion of iodine by patients with hyperthyroidism is an indication of a state of increased loss of body iodine. However, a normal urinary excretion of iodine in hyperthyroidism does not necessarily indicate that there is not a pathologic increased loss of the body iodine. It may be accompanied by an increased output of iodine through other channels, it may be a result of renal insufficiency with retention of iodine, or possibly a result of depletion of available utilizable iodine. Evidences of at least partial depletion of the stores of utilizable iodine, in patients with hyperthyroidism, are present in the decreased iodine content of the thyroid gland, the negative iodine balance, even with a normally adequate intake of iodine, and the increased retention of iodine which occurs immediately subsequent to the administration of iodine in any form. At present it is not certain whether the incipient thyroid hyperplasia precedes or follows the onset of this process of increased mobilization, utilization and excretion of iodine. However, it is possible that the thyroid hyperplasia in hyperthyroidism is secondary to the increased loss of body iodine, and that it is compensatory in an attempt to meet a continued increased demand for utilizable iodine after the store of utilizable iodine has been consumed. —I. B.

**Pituitary control of alimentary blood flow and secretion.** Cutting, W. C., E. C. Dodds, R. L. Noble and P. C. Williams, Proc. Roy. Soc. London, s.b., **123**:22. 1937.

The administration of large doses of posterior lobe extracts containing the pressor principle causes rapid, severe, but usually non-fatal destructive necrosis of the mucosa and lining epithelium of the acid-secreting region of the stomach in rabbits and many other species. These extracts will also inhibit the secretagogue action of histamine, pilocarpine, insulin, and sham-feeding in cats, the volume of gastric secretion being primarily affected. Barium chloride intravenously administered has effects similar to those of pituitary pressor substance, while other drugs and extracts of control tissues are inert. The action of secretin on the pancreas is inhibited by pituitrin. Any agent which prevents an increase in blood-flow through the glandular area will inhibit the effect of stimulants of gastric secretion, although an increase of blood-flow in itself will not initiate secretion. The gastric lesions are attributed to the action of preformed acid gastric juice on membranes which pituitrin has made ischemic and inactive. In hypophysectomized cats with gastric fistulae, the response to histamine or to sham-feeding is abnormally small, and the usual relation between volume and acidity of gastric juice is totally obscured; small hemorrhagic erosions are sometimes encountered. When only the anterior lobe is removed, such changes are not observed.—D. L. T.

**Effects of androgenic substances in hypophysectomized rats.** Cutuly, E., D. R. McCullagh and Elizabeth C. Cutuly, Am. J. Physiol. **119**:121. 1937.

Androsterone, testosterone and dihydroandrosterone benzoate were administered to hypophysectomized rats to determine their effect upon testis repair and maintenance of accessory organs. Using adult males, subcutaneous injections were begun 1 day after operation, and continued for 18 days. Androsterone was more effective than testosterone or dihydroandrosterone in maintaining the scrotum. Androsterone was more effective than testosterone on the testes, and dihydroandrosterone was ineffective. Interstitial cells showed marked atrophy in all animals. The state of the tubules varied with substance and dose. Androsterone and testosterone (1.5 mg. per day) resulted in complete maintenance of most of the tubules, with structurally normal spermatozoa. Doses of 0.45 mg. testosterone resulted in proportionately more degenerated tubules. Dihydroandrosterone was practically ineffective. Dihydroandrosterone was least effective on the accessory organs. Testosterone was more effective than androsterone. All 3 substances caused varying degrees of maintenance, but only androsterone and testosterone prevented complete tubular atrophy. The writers believe the rôle played by scrotal function in the rat to be of considerable importance; androgenic substances prevent scrotal atrophy, and thus provide

the tubules with proper environment, since the degree of tubular maintenance seemed correlated with the degree of scrotal maintenance. For this reason caution is urged in interpreting the results of selective stimulation of the germinal epithelium.—H. O. Haterius.

## PANCREAS

**Pancreas and sodium-chloride metabolism (Pankreas und Kochsalzstoffwechsel).** Adlersberg, D., and M. Wachstein, Klin. Wchnschr. **16**:85. 1937.

Pancreatectomy in dogs led to a marked fall in blood sodium and chloride, and a marked increase in urinary excretion of these elements pari passu with the development of the diabetic syndrome. In operated animals, which were kept in balance by a measured diet and insulin, withdrawal of salt from the diet led to a fall in blood chloride and a rise in blood sugar and urinary sugar. Moreover, insulin withdrawal from the experimental animals during a salt-free period of dietary regime led to much more sever hyperglycemia and glycosuria than the withdrawal during a period of salt feeding. Finally, the dogs seemed to be less sensitive to insulin in the hypochloremic state than normally. When, however, an attempt was made to apply these findings to humans, no success was encountered. Variations in the salt intake in a variety of diabetics gave no clear indication of any influence on the carbohydrate metabolism.—M. H. F.

**Blood sugar recovery from insulin hypoglycemia after section of the splanchnic nerves.** Berg, B. N., and T. F. Zucker, Am. J. Physiol. **120**:435. 1937.

The purpose of these experiments was to determine the nervous influence upon insulin hypoglycemia. Nineteen healthy male cats were used, 5 with no previous treatment, 4 after lumbar ganglionectomy, and 10 after splanchnic section. Blood sugar determinations were made by the Schaffer-Hartman method. After section of the splanchnic nerves in cats, 2.0 U of insulin per kg. always produced shock and was usually accompanied by convulsions. Blood sugar determinations corroborate this in the sense that the splanchnics carry the impulses for prevention of excessive lowering of the blood sugar, but in animals where this emergency mechanism had been abolished a spontaneous restitution of blood sugar to normal values took place. Removal of the second and third lumbar sympathetic ganglia did not produce reactions to insulin differing from the normal.—K. A. Brownell.

**Divided dosage of insulin.** Bischoff, F., and Lillian M. Jemtegaard, Am. J. Physiol. **119**: 149. 1937.

In this work a comparison was made between a certain amount of insulin given in a single dose, and the same amount divided into 4-24 parts and administered over periods ranging from 1-24 hr. The object of the experiments was to match the blood sugar curves produced by subcutaneous injection of insoluble insulin derivatives with those observed when frequent doses of regular, clinical insulin are given, and thus to obtain some clue as to the rate of liberation of insulin from the insoluble combination. Ten rabbits were used, each serving as the subject for several observations and as its own control. By injecting insulin in divided doses at regular intervals, it was possible to reproduce hypoglycemic curves resembling those obtained by injecting tissue insoluble insulin combinations. A similar intensity of hypoglycemia with prolonged effect was produced when the time of resorption extended over 3-5 hr. A more shallow hypoglycemic curve was produced when the resorption period extended over 7 hr. Hourly doses of 1/24 U of insulin per kg. or less still produced hypoglycemia.—K. A. Brownell.

**Experiences in the insulin-hypoglycemia treatment of schizophrenia.** Cameron, D. E., and R. G. Hoskins, J. A. M. A. **109**:1246. 1937.

Their year's experience leads the authors to feel that the Sakel method is a promising therapeutic approach to the schizophrenic problem. The method has not yielded the high percentage of recoveries claimed from some European centers, but since that experience appears to be a considerable factor they believe it wise to suspend judgment as to the final efficacy of the method. In view of the well-known tendency of the schizophrenia reaction to show considerable fluctuation both in form and intensity, considerable care must be exercised to distinguish between the results of treatment and the endogenous fluctuations. Apart from the value of insulin treatment in the immediate alleviation of early cases, there are many questions such as the permanence of the recoveries, the question of the most favorable kind of case, and the light this method may throw on etiology, which can be answered only after further investigation. Since the experience plays such a large rôle in determining efficacy and forestalling danger, the method should not be attempted save in an adequately equipped mental hospital, or under the immediate supervision of a well-trained psychiatrist, who should also be capable of meeting

the emergencies that may arise. Treatment has been completed in 17 cases. These patients were treated from 5 to 6 times a week over periods that ranged from 2 to 10 months. Two have apparently recovered, and 5 others have shown themselves capable of being at home for varying periods. Five patients showed no improvement. No ill effects were noted in cases in which treatment has been prolonged. When improvement does occur, the first changes consist in an increased interest in the environment. Later there are changes in the formal behavior. Relapses are frequent. Sometimes it is possible to ascribe the relapse to errors in the technic, and sometimes they appear to be due to psychologic factors as, for instance, when some one endeavors to probe into the causes of the patient's earlier breakdown while his recovery is still in its early stages.—Abst., A. M. A. Service.

**On the diastase therapy in the treatment of diabetes mellitus (Ueber die Diastasetherapie in der Behandlung des Diabetes mellitus).** Deichmann-Grubbler, W. and V. C. Myers, Biochem. Ztschr. **288**:149. 1936.

Confirming previous workers, diatase injected intraperitoneally in rabbits caused hypoglycemia, but no effect was observed after intravenous injection. Diastase had also a favorable influence on carbohydrate metabolism in fairly light cases of diabetes in humans, lowering blood sugar and having an even stronger effect on glycosuria. In different preparations of diastase the enzymatic activity did not correspond with the action on blood sugar. Of the preparations tested taka diastase had the greatest effect on blood sugar. Malt diastase, on the other hand, was the strongest enzyme but had relatively little influence on carbohydrate metabolism. It is concluded that the hypoglycemia produced by diastase is not due to enzyme action, but to a separate principle adsorbed by the enzyme.—Emil Bozler.

**Hypophyseal diabetes and pancreatic diabetes in vagotomized dogs (Diabetes hipofisiaria y diabetes pancreática en el perro vagotomizado).** Etcheverry, A. O., Rev. Soc. argent. de biol. **13**:136. 1937.

Vagotomy in 3 dogs and vagotomy with splanchnicotomy in 2 dogs did not modify the hyperglycemic and glycosuric action of APE, similar results having been obtained in 2 normal controls fed with the same diet (300 gm. meat, 50 gm. sugar daily). The same operations in 2 other pairs of dogs failed to modify the course of diabetes produced by pancreatectomy. —J. T. L.

**Effect of vagotomy on the blood sugar curve after glucose and insulin injection (Acción de la vagotomía sobre las curvas glucémicas por glucosa insulina).** Etcheverry, A. O., Rev. Soc. argent. de biol. **13**:64. 1937.

Both vagi were cut above the diaphragm in dogs, and glucose (1 gm./kg.) injected intravenously before and after the operation. The initial normal level was reached much later after vagotomy. Hypoglycemia, produced by insulin (2 U intravenously) was more marked and prolonged after vagotomy. In cats, no difference was observed in the blood sugar curves obtained before and after vagotomy, but in rabbits the return to normal was significantly slowed after hyperglycemia by glucose injection. The resting blood sugar was normal in all the vagotomized animals.—J. T. L.

**Effect of denervation of pancreas or liver and extirpation of the abdominal sympathetic on blood sugar regulation (Modificaciones de la glucorregulación determinadas por la desnervación del pedículo pancreático o del pedículo hepático, o la simpatectomía abdominal en los perros).** Etcheverry, A. O., Rev. Soc. argent. de biol. **13**:74. 1937.

Denervation of the pancreas in dogs did not modify the basal blood sugar level. Injection of glucose produced a more prolonged hyperglycemia than that observed before operation. Injection of insulin produced a more marked and prolonged hypoglycemia than before operation. Denervation of the liver did not modify the basal blood sugar, nor the blood sugar curves following insulin or glucose injection. After removal of the abdominal sympathetic chain in normal and vagotomized dogs the blood sugar curve following glucose injection showed the same characteristics as before the operation.—J. T. L.

**Stimulation of the vagus nerve and insulin secretion (Excitación de los nervios vagos y secreción de insulina).** Etcheverry, A. O., Rev. Soc. argent. de biol. **13**:130. 1937.

Under chloralose anesthesia, both adrenals in dogs were removed, the liver denervated and the splanchnic nerves cut; 2 hr. later, the vagi were stimulated for 30 min. at the level of the heart. The blood sugar started to fall before the nerve stimulation, and this last did not produce a marked hypoglycemia. To avoid the effects of shock in another series, the dogs were operated

in 3 stages, at intervals of 12 to 15 days. In the first operation, the left adrenal medulla was removed, the left splanchnic nerve cut, the left abdominal sympathetic chain extirpated, and the liver denervated. In the second operation the right adrenal and abdominal sympathetic were removed and the right splanchnic cut. In the third stage under chloralose, the vagi were stimulated above the diaphragm 2½ to 3 hr. after placing the electrodes; stimulation lasted 35 to 40 min. Blood sugar was followed half-hourly for 15 or more hr. A slight drop in glycemia was seen in some cases 1 or 2 hr. after stimulation, as also in controls not stimulated. Only 1 case out of 6 showed a marked hypoglycemia. An insulin-secretion stimulating activity of the vagus could be shown with the method used.—J. T. L.

**Increase in severity of pancreatic diabetes by anterior pituitary lobe extract (La agravación de la diabetes pancreática por el extracto alcalino del lóbulo anterior de hipófisis).** Foglia, V. G., Rebeca Gerschmann, A. D. Marenzi, J. Muñoz and C. T. Rietti, Rev. Soc. argent. de biol. **13**:83. 1937.

Pancreatectomy was performed in dogs kept on a standard diet and given 2 to 3 U insulin per kg. daily. After an interval, allowing recovery from operation, insulin in 3 (group A) was discontinued and they were given daily 5 cc. (1 gm. fresh gland) alkaline APE intraperitoneally. Another 3 (group B) continued to receive the insulin treatment, and were also given the above dose of APL extract. In a third group (C) of 4, used as controls, the insulin treatment was stopped. The controls survived 8 to 10 days, group A died on the 2nd and 3rd day of pituitary treatment, and group B on the 4th. The extract increased the severity of the diabetes, as was shown by *1)*, a very marked and early rise in acetonemia and acetonuria; *2)*, a marked increase in the fatty acids in the blood and the lipids of the liver; *3)*, an accelerated decrease in the $CO_2$-combining power, K, and accelerated increase in the inorganic phosphates of plasma. Insulin has an antagonistic effect on the activity of the extract in this respect. There was no difference in the 3 groups which showed: decrease in plasma Cl and Ca; hyperglycemia and glycosuria and no significant variation in blood cholesterol and phospholipids. The general symptoms (vomiting, diarrhoea, depression) were considerably enhanced by the pituitary extract; but the loss of weight was similar in the 3 groups.—J. T. L.

**The electroencephalogram of schizophrenics during insulin hypoglycemia and recovery.** Hoagland, H., M. A. Rubin and D. W. Cameron, Am. J. Physiol. **120**:559. 1937.

Brain wave records were obtained on 6 patients during 35 insulin treatments for schizophrenia. During the coma the alpha-waves were found to decline in frequency and large, slow, arhythmic waves appeared. The alpha-wave frequency parallels the decline in blood sugar while the longer waves bear a roughly inverse relation to the blood sugar curve. A method for measuring the longer waves is described.—R. R. Durant.

**Endocrine progress and its relation to essential hypertension and diabetes mellitus.** Hutton, J. H., Illinois M. J. **71**:469. 1937.

The author points out that the frequent association of essential hypertension with diabetes mellitus in the same patient probably indicates more than a mere coincidence; both appear to originate in a common etiological factor which may be occasioned by a functional disturbance of the pituitary, adrenals, or both. A series of case histories is reported, in which low dosage irradiation of these glands yielded improvement.—I. B.

**Insulin and gastric motility.** Lalich, J., W. B. Youmans and W. J. Meek, Am. J. Physiol. **120**:554. 1937.

Gastric inhibition by insulin in vagotomized dogs was found to persist after splanchnicotomy, removal of the coeliac ganglia and one adrenal, and demedullation of the other adrenal. The action is therefore believed to be peripheral.—R. R. Durant.

**Hyperalimentation in normal animals produced by protamine insulin.** MacKay, E. M., and J. W. Callaway, Proc. Soc. Exper. Biol. & Med. **36**:406. 1937.

The daily injection of 16 U of protamine zinc insulin into adult male rats markedly increased the food intake, and accelerated the growth curve (body weight).—M. H. F.

**The adrenals and experimental pancreatic diabetes.** Rogoff, J. M., and H. W. Ferrill, Arch. Int. Med. **60**:805. 1937.

The supposed dependence of experimental pancreatic diabetes on secretion of epinephrine from the adrenals is not supported by substantial experimental evidence. Severity of diabetes in

depancreatized dogs is not modified by reduction or suppression of the secretion of epinephrine from the adrenals. The requirement for insulin, when the diet is constant, is within the same range in depancreatized dogs as in dogs with a reduced or suppressed secretion of epinephrine in addition to pancreatectomy. Depancreatized dogs with a reduced or suppressed secretion of epinephrine are not more sensitive to insulin than ordinary depancreatized dogs. Depancreatized dogs kept for several weeks on a constant diet with adequate amounts of insulin to control glycosuria sooner or later show a reduction or suppression of epinephrine output from the adrenals. Results obtained for depancreatized animals that have been subjected to operations on the adrenals should be interpreted with caution, unless the animals are obviously in excellent physical condition. The clinical practice of intervention with the adrenal glands for the relief of diabetes (or other conditions, except unilateral malignant neoplasm) does not rest on unequivocal experimental premises, and it should be deprecated as too dangerous for life and health.—I. B.

**The hypoglycemic treatment of schizophrenia: A preliminary report, with particular reference to the qualitative study of remissions.** Rymer, C. A., J. D. Benjamin and F. G. Ebaugh, J. A. M. A. **109**:1249. 1937.

In presenting a preliminary report of their experiences with the hypoglycemic treatment of schizophrenic reactions, the authors discuss the methods of study employed, as well as the actual results obtained, in their series of 7 completed cases. They feel that the results of the hypoglycemic treatment of schizophrenia to date (excellent results were obtained in 5 of their 7 patients) are highly encouraging, although a long period of time will be necessary before a definite opinion can be given. Apart from its therapeutic value, the method offers an unusual opportunity for clinical and laboratory research. The most promising approach at the present time appears to them to lie in the qualitative study of remissions; not only whether the insulin treatment increases the number of remissions, but also whether these are in any way better than or different from spontaneous remissions.—Abst., A. M. A. Service.

**The influence of insulin on the complement content and its relation to the healing of wounds in surgery (Azione dell' insulina sul potere complementare in rapporto alla guarigione delle ferite chirurgiche).** Scartozzi, C. Arch. per le sc. med. **62**:495. 1936.

Insulin treatment increases the complement content in the guinea pig's blood. If given previous to the operation, it exerts a favorable influence on the healing process of wounds caused by laparotomy. It is without effect if given after the operation.—A. E. Meyer.

**The insulin-sparing action of copper (Über eine insulinsparende Wirkung des Kupfers).** Schnetz., H., Klin. Wchnschr. **16**:664. 1937.

The administration of 10 to 20 mg. of copper sulphate orally to patients without metabolic disturbances decreased the usual hyperglycemic response to subcutaneously injected adrenalin (0.5 mg.) and the alimentary hyperglycemia following the ingestion of 30 gm. of dextrose. These experiments on man confirm the work of others on rats and rabbits in regard to the effect of copper on adrenalin and alimentary hyperglycemia. Several patients with severe diabetes were given 20 mg. of copper sulphate daily, which resulted in a diminished insulin requirement and improved clinical condition. In a case of mild diabetes which required 30 U of insulin per day, the administration of copper permitted the discontinuance of insulin altogether. —M. H. F.

**Normoglycemic glycosuria differentiated from other benign glycosurias and diabetes mellitus.** Smith, Florence H., and K. A. Smith, Arch. Int. Med. **60**:119. 1937.

This paper presents a review of 3 cases of glycosuria in which the sugar content of the blood was normal. The authors feel that attention should be called to the necessity of a complete investigation of all cases of glycosuria before the institution of the rigid management required by the diagnosis of diabetes mellitus. The social and financial implications of this disease are well realized, even by one not in frequent contact with diabetic patients. For these reasons alone the diagnosis should be made only after the most careful study, and the accuracy of the diagnosis should be suspected if, after a sufficient trial, careful management is unsuccessful. Of the nondiabetic glycosurias, that due to a lowered renal threshold for dextrose is the most common; the others, such as pentosuria, lactosuria, fructosuria and galactosuria, are referred to briefly. A simple but adequate method of establishing a diagnosis is presented. This method consists of frequent analyses of the sugar content of the blood by the microcolorimetric method of Folin and Svedberg, and simultaneous urinalyses before and after a dextrose meal. The use

of the Woodyatt forcing diet preceding the dextrose tolerance tests, as a means of excluding mild diabetes mellitus, is demonstrated.—I. B.

## PARATHYROIDS

**Two cases of hyperparathyroidism.** Fahrni, G. S., Canad. M. A. J. 37:11. 1937.

Two cases with successful removal of parathyroid adenomata; the first notable for a series of skiagrams illustrating the progress of the disease.—D. L. T.

**On the calcium content of different parts of the brain of normal and thyroid-parathyroidectomized rats (Ueber den Calciumgehalt verschiedener Teile des Gehirns von normalen und thyreoparathyreoidektomierten Ratten).** Farage, S., Biochem. Ztschr. 288:393. 1937.

In thyroid-parathyroidectomized rats, Ca was increased in the hemispheres and cerebellum and diminished in the medulla. Parathyroid extract tended to re-establish the normal relation of the Ca content. Thyroxine, however, did not change the Ca content of the various parts of the brain in operated animals.—E. Bozler.

**Electrocardiogram after total parathyroidectomy (Modificaciones electrocardiográficas consecutivas a la paratiroidectomía total).** Segura, A. S., Rev. Soc. argent. de biol. 13: 170. 1937.

Thyro-parathyroidectomy was performed in dogs; the electrocardiogram (mouth-anus derivation) was taken before and on the days following operation, the animals were not anesthetized. Thyroidectomized controls were made. T-parathyroidectomy produced modifications due to a diminished vagus tone: tachycardia, disappearance of respiratory arhythmia, diminished auricular-ventricular conduction time (P-Q interval shortened), these could be reproduced by atropine injection. The electric systole (Q-T interval) was prolonged; this was not due to decreased vagal tone. Irregular alterations in the T wave and in the ST segment similar to those observed in insufficient coronary circulation were recorded. Calcium treatment re-established a normal E. C. G.—J. T. L.

**Magnesium metabolism in health and disease. I. The magnesium and calcium excretion of normal individuals, also the effects of magnesium, chloride, and phosphate ions. II. The effect of the parathyroid hormone. III. In exophthalmic goitre, basophilic adenoma, Addison's disease, and steatorrhea.** Tibbetts, D. M., and J. C. Aub, J. Clin. Investigation 16:491, 503, 511. 1937.

Methods for magnesium-balance determination are given, and studies on 10 different individuals are reported to show that balance is attained at levels from 220 to 300 mg. magnesium daily. There is a certain parallelism between the calcium and magnesium responses to ammonium chloride and phosphate ingestion, but the results suggest that there is magnesium mobilized from soft tissues as well as from bone. The effects of hyperparathyroidism and its relief on magnesium excretion are qualitatively similar to those of calcium, but much less marked. Magnesium cannot be substituted for calcium in the excretion, but possibly can substitute in part in the bones. In the study of thyrotoxicosis, basophilic adenoma, Addison's disease, and steatorrhea (1 case each) there was no evidence that the patients had much disturbance of magnesium excretion, although they had marked alteration of balances of the other kations.—E. L. S.

## THYMUS

**The thymus in health and disease.** Michael, P., California & West. Med. 46:101. 1937.

Two hundred case histories are summarized in which the thymus was studied. Birth weights agreed with those of other observers, but puberty weights and adult weights were much less than those generally given.—I. B.

## THYROID

**On the occurrence of a substance containing iodin and antagonistic to thyroxin in the thyroid (Ueber das Vorkommen einer jodhaltigen, antithyroxinartig wirkenden Substanz in der Schilddrüse).** Abelin, I., Biochem. Ztschr. 286:160. 1936.

A peptone containing iodin was isolated from hydrolyzed thyroid tissue. The substance, administered orally, lowers the basal metabolic rate and produces a general improvement in

rats during experimental hyperthyroidism. Iodin as peptone kept the B.M.R. at about +50%, whereas rise to about 100% was found in the controls. Hyperthyroidism was not diminished by inorganic iodin or diiodotyronine. On decomposition of the peptone diiodotyrosine was formed. It is concluded that the thyroid produces besides thyroxin a related substance which acts as antagonist to the former.—E. Bozler.

**Reticulocytes in endocrine disease with special reference to exophthalmic goiter (Retikulotsityj pri endokrinnyjkh zabolevanijakh V Chastnostic pri bazedovoi balezni).** Althausen, A. J., Acta Endocrinologica Klinika endokrinnyjkh sabolevanii 9:230. 1936.

Reticulocyte counts were made on capillary blood of 232 patients, classified into 21 endocrine groups. A reticulocyte count of 1 to 4% was considered as normal, above 5% as high. Questionable reticulocytosis was found in diabetes mellitus and insipidus. A considerable elevation of reticulocytes was found in acromegaly (3 cases) adrenal cortical hyperplasia (2 cases), Addison's disease (4 cases) and in the exophthalmic goiter (85 cases). In the latter condition, reticulocytosis roughly paralleled the gravity of the condition. Operation was followed by a lowering of reticulocytes to levels close to normalcy within several months to a year.—B. N. Tager.

**Choline-esterase activity of human sera with special reference to hyperthyroidism.** Antopol, W., L. Tuchman and A. Schifrin, Proc. Soc. Exper. Biol. & Med. 36:46. 1937.

Determinations of the acetyl-choline esterase content of the blood by a gasometric technic (carbon-dioxide measurement after its liberation from carbonate solution during the hydrolysis of the ester), conducted on 60 control patients and on 22 untreated cases of hyperthyroidism revealed the latter to have a significantly higher amount of the enzyme. However, some patients with diabetes or hypertension also showed elevated values.—M. H. F.

**On the biologic estimation of the thyro-stimulin of the anterior lobe of the hypophysis: The mitotic threshold in thyroids of treated guinea pigs (Sur le titrage biologique de la thyréo-stimuline préhypophysaire: Le "seuil des mitoses" dans la thyroïde des cobayes traités).** Aron, M., Compt. rend. Soc. de biol. 123:250. 1936.

In measuring the potency of a preparation, 100 U is considered that amount of thyrostimulin which will produce in a young guinea pig (200 gm.) after 24-36 hours an increase of thyroid weight to 50-100% above the average, together with total excretion of colloid in the center of the gland and signs of hypersecretion throughout the entire organ. On this basis, 10 U produce evidence of hyperactivity in the center of the gland only. Mitotic activity does not show any increase until about 40-50 U are given. With larger amounts, however, it is roughly proportional to the dose.—J. C. D.

**Blood sugar and blood cholesterol after thyroid resection (Über das verhalten des blutzuckers und des blutcholesterins nach schilddrüsenresektion).** Atnam, A., E. Fenz and K. Uiberrak, Wien. Arch. f. inn. Med. 31:23. 1937.

The blood sugar, blood cholesterol and blood iodine were studied before and after various operations, including appendectomy, cholecystectomy and thyroid resection. Following operations on the thyroid gland, the postoperative elevation of blood sugar tended to persist, frequently for as long as 24 hr. This was in contrast to the effects observed following operations in other localities, for in these cases the blood sugar fell to normal in 3 to 5 hr. The persistent hyperglycemia following thyroidectomy is not dependent on the type of anesthesia or the variety of thyroid disease. It is assumed that during the operation an excess of thyroid secretion is pressed out and enters the blood stream. Following operations in other localities, little or no depression of blood cholesterol could be observed, whereas after thyroid resection there was a definite fall. In 2 cases it was observed that the blood iodine showed double the normal value for a short time after resection of the thyroid.—W. J. A.

**Glutathione and thyroid (Glutation y tiroides).** Beaux, A. R., Tesis, Buenos Aires, 1935. Abst., Physiol. Absts. 22:410. 1937.

Braier and Marenzi's method was used for the estimation of reduced glutathione. In patients with hyperthyroidism the quantity of glutathione contained in the red cells is lower than the average normal value. After therapeutic thyroidectomy there is a slow and gradual increase of blood glutathione. In dogs with hyperthyroidism, provoked by ingestion of dried thyroid glands, the blood glutathione decreases; after thyroidectomy it rises above the initial

value. In thyroidectomized dogs the ingestion of thyroid brings the blood glutathione nearly to its initial value.

**Part played by the thyroid in gonad stimulation by artificial light in domestic ducks (Rôle de la thyroïde dans la gonado-stimulation par la lumière artificielle chez le canard domestique).** Benoit, J., Compt. rend. Soc. de biol. **123**:243. 1936.

Nine thyroidectomized and 3 control ducks were exposed to the same dosage of artificial light. The stimulation of testicular growth was much less marked in the operated than in the control birds.—J. C. D.

**The glutathione in the blood and tissues in relation to thyroid function. (El glutatión sanguíneo y tisular en la función tiroidea).** Braier, B., Semana méd. **44**:289. 1937.

Subjects with elevated metabolism show a reduction in the blood glutathione. Thyroid treatment in rats, guinea pigs and rabbits caused a drop in blood glutathione of 20% concomitant with an increase in the tissue of heart, liver, lungs, spleen and testes. There was no change in the kidneys and muscle tissue.—A. E. Meyer.

**Studies on the physiology of the thyroid. IV. Goitre-Basedow's Disease (Studien zur Physiologie der Schilddrüse. IV. Kropfnoxe-Basedownonoxe).** von Blum, F., Endokrinologie **19**:19. 1937.

The author confirms previous findings that the feeding of certain varieties of cabbage to rabbits is followed by goiter and exophthalmos. In pregnant animals, to which the cabbage was fed, the noxious substance traversed the placenta and produced goiters in the fetuses. The possible existence of different substances which act on the thyroid, some to produce exophthalmic goiter and others cretinism, is discussed.—W. J. A.

**Relation of thyroid and parathyroid glands to calcium and phosphorus metabolism. Study of a case with coexistent hypoparathyroidism and hyperthyroidism.** Cope, O., and G. A. Donaldson, J. Clin. Investigation **16**:329. 1937.

When the basal rate was maintained within normal limits by iodin therapy, the calcium and phosphorus balances were characteristic of those seen in parathyroid tetany. With hyperthyroidism the negative balance of calcium and phosphorus increased markedly in spite of decreased signs of parathyroid activity. The increasing thyroid activity increased serum calcium and relieved the tetany. The findings give support to the belief that the changes in calcium metabolism known to occur in hyperthyroidism are not due to concomitant hyperparathyroidism. —E. L. S.

**The fallacy of the use of iodine immediately after bilateral subtotal thyroidectomy.** Davison, M., and L. J. Aries, Surg. Gynec. & Obst. **64**:999. 1937.

One hundred consecutive patients with hyperthyroidism were studied. In all the patients, the classical operation of bilateral subtotal thyroidectomy was done in one stage. They were then divided in two groups. Group A, 50 patients were given Lugol's solution after operation, Group B, 50 patients were untreated after operation. The postoperative course was judged by temperature, pulse, respiration and general toxicity of the patient on the basis of the B.M.R. It was found that patients in the Group B series (untreated) had milder reactions, and that patients in Group A developing the operative toxic phenomena were not influenced by iodine medication. In general, if sufficient gland has been removed, and if the patient has been adequately prepared before operation, the use of post-operative iodine medication seems to have no rational basis.—L. B. S.

**Total thyroidectomy for human diabetes insipidus.** Findley, T., Jr., and P. Heinbecker, Proc. Soc. Exper. Biol. & Med. **36**:448. 1937.

The urine output in a case of diabetes insipidus was unchanged by total thyroidectomy, but salt administration caused less, and pitressin more, of a decrease after the operation than before.—M. H. F.

**Effect of midbrain narcosis in Basedow's disease (Über die Wirkung der Zwischenhirnnarkose bei Morbus Basedow).** Fenz, E., Wien. Arch. f. inn. Med. **30**:15. 1937.

Eight normal persons and 14 with Basedow's disease or with hyperthyroidism were held in deep narcosis ('midbrain narcosis') with large doses of veronal for 2 or 3 days or with

prominal for 3 to 7 days. In this condition normal persons showed a moderate lowering of the basal metabolic rate (6 to 18%), a lowering of the blood cholesterol and blood carotine and no change in the alkali reserve. Patients with Basedow's disease showed a marked lowering of the B. M. R. (as much as 54%) with distinct improvement in the clinical signs and symptoms. It is held probable that there had been a reduction of thyroxin production through depression of the thyroid-regulating center of the midbrain.—W. J. A.

**Effect of midbrain narcosis on blood iodin in Basedow's disease (Über die Wirkung der Zwischenhirnnarkose auf das Blutjod bei Morbus Basedow).** Fenz, E., and K. Uiberrak, Wien. Arch. f. inn. Med. **30**:135. 1937.

Midbrain narcosis in 16 cases of Basedow's disease or of hyperthyroidism produced a more or less distinct, often prolonged, lowering of the blood iodin. In 3 persons with normal metabolism midbrain narcosis produced only slight lowering of the blood iodin; in a case of myxoedema there was no effect. The fall of blood iodin during midbrain narcosis in cases of Basedow's disease was greatest in those which showed the highest blood iodin prior to narcosis. No definite correlation between elevation of the basal metabolic rate and blood iodin value could be made. The explanation offered for the fall of blood iodin is that midbrain narcosis causes a diminution of the production of thyroid secretion.—W. J. A.

**Effects of thyroid therapy on the mental and physical growth of cretinous infants.** Gisell, A., C. S. Altruda and C. S. Culatta, Am. J. Dis. Child. **52**:1117. 1936.

This is a report on the findings in 6 patients with cretinism or hypothyroidism in which the condition was recognized and adequately treated in infancy. All were girls, the youngest being 21 weeks at the time of diagnosis. The mental retardation as measured by the developmental quotient ranged from 10 to 85 points below 100. Improvement in mental status ranged from 15 to 65 points. The duration of therapy was from 1 to 10 years. The effects of desiccated thyroid substance on the cretinous infant are of three kinds: metabolic; somatic; and neurodevelopmental. The metabolic effects are the most immediate and general. Marked physical improvement occurred in all the cases. The final influence is contingent not so much on the age of the child or maturity status at the time of diagnosis as on the residual physiological capacity and latent growth potency of the neuro-endocrine system. Thyroid therapy cannot bring about normality if there has been a fundamental impairment of that system, hereditary or developmental.—M. B. G.

**The prevention of goitre in Wisconsin: A challenge to the medical profession.** Jacobson, A. S., Wisconsin M. J. **35**:15. 1936.

One out of every 5 school children in Wisconsin shows evidence of disturbance of thyroid. Among 1000 children who have had iodin for 12 years, adenomatous goiter has not developed if it had not previously existed. The author feels that thyroid extract plus iodin salt is required.—M. B. G.

**The lysine treatment of Basedow's disease (La lasatotherapie de la maladie de Basedow).** Kasakow, I. N., Acta med. Scandinav. **92**:135. 1937.

A poly-lysine is prepared by hydrolyzing separately the hypophysis, parathyroid, adrenal cortex, liver, muscles, gonads, pancreas and spleen, with weak sulphuric acid in the autoclave and making a mixture of these with brain lipoids. It consists of polypeptids of low molecular weight, amino acids, lipids and diketopiperazines and was given intramuscularly in 700 cases of Basedow's disease. In 435 cases the blood pressure fell after treatment and the pulse rate returned to normal. The exophthalmos disappeared in 25.5% of the cases, diminished in 72.1% and remained unchanged in 2.4%. The B. M. R. was reduced in a large proportion of the cases.—J. M. L.

**A Study of cretinism in London. With special reference to mental development and problems of growth.** Lewis, A., N. Samuel and J. Galloway, Lancet **232**:505. 1937. Lancet **233**:5. 1937.

The authors undertook an investigation of the psychological effects of thyroid deficiency in childhood, and the benefits derived from substitution therapy on mental retardation. This study was carried out on 79 patients. Detailed psychological tests showed that it is possible for cretins to become mentally normal. In the majority of instances there was a rough correlation between the adequacy of treatment and intellectual level attained. Other factors which

influence psychological development are apparent stage of development at which symptoms of thyroid deficiency first appear, the degree of this deficiency, hereditary endowment of the child, cerebral damage at birth or infancy, and the environmental influences.—L. B. S.

**Early effect of total thyroidectomy in a case of polycythemia vera (Vaquez Osler syndrome).** Limarzi, L. R., R. W. Keeton and L. Seed, Proc. Soc. Exper. Biol. & Med. 36:353. 1937.

In a patient with the classical blood picture, signs, and symptoms of polycythemia vera, a total thyroidectomy induced alterations in the blood picture which were in the direction of normality, especially the total plasma volume, and also relief from the symptoms of the syndrome. However, the operation did result in some of the symptoms of myxedema.—M. H. F.

**Investigations on the treatment of thyreotoxicosis.** Meyler, L., Acta Med. Scandinav. 93:48. 1937.

The author discussed the unfavorable effect of intercurrent infection on thyreotoxicosis. In 27 of 30 cases of exophthalmic goiter there was a disturbance of liver function as indicated by the urobilinuria and decreased galactose tolerance. After operation in 9 patients there was an improvement in liver function, as shown by the test. In 16 patients a lowering of the B.M.R. followed the giving of diets containing 800 to 1000 calories and 20 gm. of protid per day. The creatinuria was markedly decreased in 6 patients on this diet.—J. M. L.

**The endocrine system and plumage types: some effects of hypothyroidism.** Parkes, A. S., and H. Selye, J. Genetics 34:297. 1937.

Thyroidectomy has been performed on fowls of different breeds. Feathers which grow during hypothyroidism are elongated, and show loss of barbules leading to fringing. Color changes vary with the breed; the deposition of black pigment is restricted in the Brown Leghorn and Silver Dorking, but extended in the Ancona.—D. L. T.

**Effect of thyroid feeding on the excretion of morphine.** Plant, O. H., and D. Slaughter, J. Pharmacol. & Exper. Therap. 60:116. 1937.

In the non-tolerant dog, the average daily excretion of morphine was decreased 12.6% by thyroid administration (4 gm. per day for 3 days), whereas in the tolerant animal it was increased 21%. The authors believe that the diuresis produced in the tolerant animals was a factor in the increased excretion.—E. L.

**New methods of diagnosis and treatment of thyroid disease.** Poer, D. H., South. M. J. 30:474. 1937.

In this general discussion, tables are given of a group of cases studied by the author. These include 17 hyperthyroid cases, 16 of endemic goiter, and 7 of non-thyroid conditions. The basal metabolic rate and the blood iodin level are given. The author emphasizes the importance of the blood iodin as a more accurate indicator of the thyroid activity than the basal metabolism test alone.—J. C. D.

**Specific dynamic action of foods in exophthalmic goiter (Spetsificheski-dinamicheskoe deistvie ingredientov pischi pri bazedovnoi bolezni).** Reznitskaija, E. J., Acta Endocrinologika, (Klinika Endokrinnyjkh zabolevanii) 9:199. 1936.

The authors demonstrate marked specific dynamic effect of food in hyperthyroidism. In 60 cases studied (B.M.R. ranging from +16 to +92%), the response to carbohydrate was an increment of 55%, to proteins 60%, and to fats 46% over initial values. Responses were not proportional to initial B.M.R. levels. Sugar was administered in the form of 100 cc. of 50% glucose, and B.M.R. readings were taken 40, 80 and 150 minutes after ingestion. Protein was administered at 100 cc. of a 20% peptone solution, and B.M.R. readings were taken 1, 2 and 3½ hours after ingestion. Fat was administered in form of 75 gm. of butter on 20 gm. of bread, and B.M.R. tracings taken 3, 6 and 9 hours after ingestion. In 20 normal controls no significant changes in the B.M.R. could be observed.—B. N. Tager.

**Immunity to iodothyroglobulin does not affect its physiological action.** Rosen, S. H., and D. Marine, Am. J. Physiol. 120:121. 1937.

Five adult rabbits (4 males and 1 female) were repeatedly injected intraperitoneally 11 to 28 times triweekly with iodothyroglobulin, a highly antigenic substance. Measurements

of heat production were followed, and precipitin titrations on the blood with iodothyroglobulin were performed. No decrease in physiological response (increased heat production) was observed on repeated injections, despite the small but definite precipitin response. "These observations confirm the opinion that thyroxine is not a part immunologically of the iodothyroglobulin molecule."—Lena Lewis.

**Anatomical control of iodine prophylaxis of endemic goitre (Beitrag zur anatomischen Kontrolle der Jodprophylaxe des endemischen Kropfes).** Schnetz, A., Endokrinologie **19:**164. 1937.

From many autopsy records in Switzerland a statistical study has been made of the anatomical condition of the thyroid gland in the years before and after the application of iodine prophylaxis for endemic goiter. A statistically significant decrease in the percentage of diffuse and nodular goiters is found in the period of iodine prophylaxis. It is shown that this treatment is most effective in childhood and youth.—W. J. A.

**Assay of thyroid U.S.P. by determination of oxygen consumption in the guinea pig.** Shackell, L. F., J. Pharmacol. & Exper. Therap. **60:**117. 1937.

Thyroid iodin, 0.1 to 0.2 mg. per kg., given orally as desiccated thyroid, causes an increase in $O_2$ consumption of 10 to 20% which persists for about 3 days. When this dosage is given hypodermically as thyroxin, the $O_2$ consumption increases 15 to 25% and persists for about 4 days. The author believes that thyroid U.S.P. should be equivalent in activity to not less than 1.8 nor more than 2.2 mg. of thyroxin assayed by measuring the oxygen consumption in the guinea pig.—E. L.

**Isotransplantation of thyroid glands in dogs.** Shafiroff, B. G. P., and K. Leora McCloskey, J. Lab. & Clin. Med. **22:**553. 1937.

Eighteen dogs were divided into 9 pairs so that the blood of each dog was of the same group as his pair mate. The thyroid glands from each dog were then transplanted into the other of the pair, one gland being put into the omentum and the other into the abdominal well. All transplants were absorbed by the end of 90 days as determined by autopsy. The B.M.R. were not lowered by the procedure. This was attributed to the constant presence of accessory thyroid nodules along the arch of the aorta or in the mediastinum.—F. A.

**The anemia of myxedema: its classification and treatment.** Sharpe, J. C., Am. J. M. Sc. **194:**382. 1937.

The literature is discussed and 5 cases are reported. In addition to the usual physical signs of myxedema, the anemic patient may present such clinical features as a lemon-yellow pallor, glossitis, paresthesias, and an achlorhydria. An erroneous diagnosis of pernicious anemia may be made. In 3 cases, the mean corpuscular volume and the hemoglobin content of the red blood cells showed that the anemia was normocytic and normochromic in character. In a fourth case an achlorhydria, hydria, hypochromia and microcytosis may have been due to an associated iron-deficiency anemia. Though thyroid extract was found to cause a prompt increase in the basal metabolic rate, a transient decrease in the blood count may first be noted, followed by a slow, and sustained rise in both hemoglobin and erythrocytes. In the presence of hypochromia, iron may accelerate the regeneration of blood cells, but liver extract appears to be of no value in this type of anemia.—R. G. H.

**The effect of heating with alkali on the calorigenic activity of dessicated thyroid and of thyroxine.** Thompson, W. O., P. K. Thompson, S. G. Taylor and L. F. N. Dickie, J. Clin. Investigation **16:**479. 1937.

The thyroid materials are given as single doses to human myxedema patients, and the changes in basal metabolism studied graphically. Using both animal and human thyroid tissues, it is shown that heating with alkali causes a significantly large loss of activity (3/5 to 4/5) but that either the heat or the alkali treatment separately do not cause this loss. The results bear on the methods used for isolation of thyroxin from the gland, and on standardization methods.—E. L. S.